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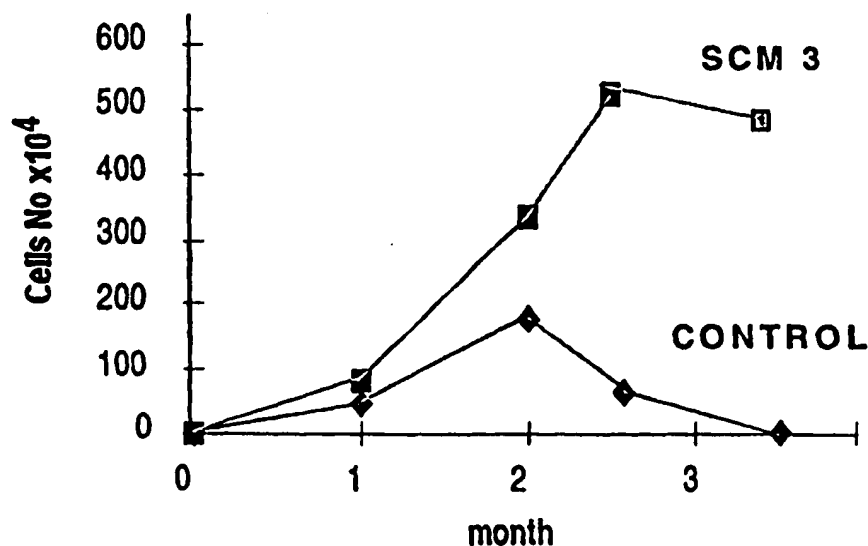
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(54) Title: NOVEL GENES IN CONTROL OF HEMATOPOIESIS



(57) Abstract

The present invention provides three novel HSC genes designated SCM 3, SCM 26 and SCM 113, the coding regions thereof, the gene products, applications of said genes, DNA constructs, vectors and transformed cells each comprising the gene or a fragment thereof. Methods of using the SCM 3, SCM 26 and SCM 113 polynucleotide and polypeptide sequences are also disclosed.

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### Novel Genes in Control of Hematopoiesis

The present invention relates to nucleic acid and amino acid sequences of three novel genes involved in the control of hematopoiesis.

Hematopoiesis (used interchangeably with hemopoiesis) is a process whereby multi-potent stem cells give rise to lineage-restricted progeny. Hemopoietic stem cells (HSCs) are the only cells in the hematopoietic system that produce other stem cells and give rise to the entire range of blood and immune system cells. In humans, CD34<sup>+</sup> Thy-1<sup>+</sup> Lin<sup>-</sup> cells from bone marrow and mobilized peripheral blood are highly enriched for HSCs (Murray et al., *Blood Cells*, 20:354 -370 (1995a); Murray et al., *Blood*, 85:368 - 378 (1995b)). This cell population is capable of self-renewal and long term multilineage differentiation and has been successfully used for autologous transplantation (Gazitt et al., *Blood*, 86:381 - 389 (1995)). Since HSCs self-renew and are multi-potent, they are ideal candidates for gene therapy. Gene therapy is a new treatment modality for a variety of genetic, neoplastic, or infectious diseases and has the potential to correct defects in all mature cells derived from HSC.

The molecular basis of hematopoiesis remains poorly understood. A greater understanding of the process whereby HSCs give rise to lineage-restricted progeny would facilitate the exploitation of HSCs for transplantation and gene therapy. To achieve this, the molecular pathways controlling hemopoietic cell growth and differentiation have been investigated. To this end, the present invention concerns the identification of candidate HSC regulatory genes and their impact on hematopoiesis.

In the present invention, a cDNA library is built and characterized from human CD34<sup>+</sup>Thy-1<sup>+</sup> stem cells. Sequence analysis of the cDNA library revealed a high degree of novel proteins which may play a role in hematopoiesis. To enrich for candidate hemopoietic stem cell (HSC) regulatory genes, RNA expression profiling is performed and cDNAs whose expression is enriched in HSC are selected and compared to other differentiated blood cell types. Candidate genes are fully sequenced. The HSC-enriched genes which encode full length novel gene products are subcloned into a retroviral expression vector, which is used to overexpress the gene product in freshly isolated HSCs. Three novel HSC regulatory genes and the proteins which they encode are identified. Each of the novel cDNAs are

enriched in HSCs compared to their differentiated progeny, and when overexpressed in HSCs blocks the differentiation of stem cells.

The present invention discloses three novel genes involved in HSC regulation, hereinafter referred to as SCM 26, SCM 3, and SCM 113.

In a first embodiment, the invention concerns isolated polynucleotide sequences encoding a polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO. 2; the amino acid sequence of SEQ ID NO. 4; the amino acid sequence of residues 1 - 239 of SEQ ID NO.4; the amino acid sequence of residues 240 - 543 of SEQ ID NO. 4; the amino acid sequence of SEQ ID NO. 6; and an amino acid sequence functionally equivalent to the above enumerated sequences.

In a second embodiment, the invention relates to an isolated DNA sequence comprising a nucleotide sequence selected from the group consisting of the polynucleotide sequence of SEQ ID NO 1; the polynucleotide sequence of SEQ ID NO. 3; the polynucleotide sequence of SEQ ID NO. 5; and a polynucleotide sequence at least 85% identical to a polynucleotide sequence as disclosed above. In one aspect, the isolated polynucleotide sequence may consist of the complement of the polynucleotide sequences of those listed above.

In a third embodiment, the invention concerns, an isolated polypeptide comprising a member selected from the group consisting of, a polypeptide comprising the amino acid sequence of SEQ ID NO. 2; a polypeptide comprising the amino acid residues 26 - 40 of SEQ ID NO. 2; a polypeptide comprising the amino acid residues 25 - 82 of SEQ ID NO. 2; a polypeptide comprising the amino acid residues 147 - 157 of SEQ ID NO. 2; a polypeptide comprising the amino acid residues 266 - 275 of SEQ ID NO. 2; a polypeptide comprising the amino acid sequence of SEQ ID NO. 4; a polypeptide comprising the amino acid residues 1 - 239 of SEQ ID NO. 4; a polypeptide comprising the amino acid residues 240 - 543 of SEQ ID NO. 4; a polypeptide comprising the amino acid sequence of SEQ ID NO. 6; or a polypeptide having at least 85% identity to said polypeptides disclosed above. In a preferred aspect, the isolated polypeptide comprises the amino acid sequence of SEQ ID Nos. 2, 4, 6, or a polypeptide having an amino acid sequence with 95% identity thereto. In another aspect, the invention relates to an isolated polypeptide comprising a member

selected from the group consisting of the amino acid residues 26 - 40 of SEQ ID NO. 2; the amino acid residues 25 - 82 of SEQ ID NO. 2; the amino acid residues 147 - 157 of SEQ ID NO. 2; the amino acid residues 266 - 275 of SEQ ID NO. 2; and a polypeptide having at least 97% identity thereto. In yet a further aspect, the invention relates to a DNA sequence encoding one of the above enumerated polypeptides.

In a fourth embodiment, the invention concerns a vector which incorporates one of the claimed polynucleotide sequences of the invention. In a preferred aspect, the vector is a retroviral vector, adenoviral vector, or adeno-associated vector. In a further preferred aspect, a host cell is claimed which includes the vector. A preferred host cell is a hematopoietic cell, particularly a human CD34<sup>+</sup> cell.

In a fifth embodiment, the invention concerns a method of increasing the effective dose of hematopoietic stem cells in a mammalian subject, comprising obtaining a population of CD34<sup>+</sup> cells which includes a subpopulation of hematopoietic stem cells; introducing into the CD34<sup>+</sup> cells a polynucleotide sequence of the invention encoding a disclosed polypeptide of the invention; obtaining a subpopulation of genetically modified stem cells which overexpress said polypeptide; and administering said subpopulation of genetically modified cells to a subject wherein the effective dose of the hematopoietic stem cells is increased. In a further aspect, the invention includes the step of selecting hematopoietic stem cells either prior to or after genetic modification. In yet another aspect, the invention includes the step of culturing the population of hematopoietic CD34<sup>+</sup> cells either prior to or after genetic modification.

In a sixth embodiment the invention concerns, a method of increasing the effective dose of gene modified cells comprising obtaining a population of hematopoietic CD34<sup>+</sup> cells which includes a subpopulation of hematopoietic stem cells; introducing into the population of CD34<sup>+</sup> cells a polynucleotide sequence of the invention; introducing into the population of CD34<sup>+</sup> cells a second polynucleotide sequence wherein said second polynucleotide sequence encodes a therapeutic gene; obtaining genetically modified cells wherein said cells are capable of expressing the polynucleotide sequence of the invention and the therapeutic gene wherein the effective dose of the cells capable of expressing the

therapeutic gene is increased compared to wild-type cells; and administering the genetically modified cells to a mammalian subject.

In an seventh embodiment, the invention concerns a method of blocking the differentiation of mammalian hematopoietic stem cells in vitro comprising the steps of: isolating CD34<sup>+</sup> cells from a source of hematopoietic cells; introducing a vector comprising the claimed polynucleotide sequences into the CD34<sup>+</sup> cells, whereby a population of said cells are genetically modified by said vector; culturing the modified CD34<sup>+</sup> cells in the presence of at least one cytokine in an amount sufficient to support growth of the modified cells; and selecting cells in which the polypeptide is overexpressed whereby differentiation is blocked. In a preferred aspect, the mammalian hematopoietic cells are human. In another preferred aspect, the CD34<sup>+</sup> cells are further selected based on the following phenotypes Thy-1<sup>+</sup>, CD34<sup>+</sup>Thy-1<sup>+</sup>, CD34<sup>+</sup>Thy-1<sup>+</sup>Lin<sup>-</sup>, or CD34<sup>+</sup>Thy-1<sup>+</sup>CD38<sup>-</sup>, either prior to or after introduction of the vector. In a preferred aspect, the method of blocking differentiation of mammalian hematopoietic stem cells includes introducing a vector comprising a claimed polynucleotide sequence into CD34<sup>+</sup> cells, genetically modifying a population of said cells with said sequence; allowing the expression of the polynucleotide sequence in said cells and blocking differentiation. The method may be in vitro or in vivo.

In an eighth embodiment, the invention concerns, a method of producing a polypeptide of the invention comprising the steps of: culturing a host cell comprising a polynucleotide sequence encoding the claimed polypeptides under conditions suitable for the expression of the polypeptide, and recovering said polypeptide from the host culture.

In an ninth embodiment, the invention relates to an antibody which binds to the claimed polypeptides. Additionally, the invention concerns a method of identifying mammalian hematopoietic stem cells or progeny thereof comprising, preparing an antibody to a claimed polypeptide; purifying the antibody; exposing a population of the mammalian hematopoietic cells to the antibody; allowing said cells to bind to the antibody; and selecting said bound cells.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and

patent applications cited herein are hereby incorporated by reference in their entirety. Throughout this specification, the singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise.

Other objects, features, advantages and aspects of the present invention will become apparent to those skilled in the art from the following description. It should be understood however, that the following description and the specific examples, while indicating preferred embodiments of the invention are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the disclosure.

#### Brief description of the drawings

The following figures depict certain embodiments of the invention. They are illustrative only and do not limit the invention otherwise disclosed herein.

Figure 1 illustrates the MIE vector. cDNAs (large arrow) are inserted into the polylinker. Transcription is driven from the 5'LTR. EGFP is included as a selective marker.

Figure 2 illustrates the nucleotide sequence and predicted amino acid sequence of SCM 26 and corresponds to SEQ ID NO. 1. The cDNA insert in clone SCM 26 is 1316 nucleotides in length and includes a polyA<sup>+</sup> tail of 18 residues. There is a single long open reading frame of 345 amino acids starting from the first in frame methionine at position 51 and ending with a TGA stop codon at position 1086. SCM 26 encodes a putative signal peptide sequence and seven transmembrane domains giving a cell surface location with an extracellular amino terminus and an intracellular COOH terminus as represented in Figure 3. The northern blot analysis indicates two SCM 26 transcripts. One transcript is 1.5Kb, and the second transcript is 2.4Kb. The cDNA clone as sequenced herein corresponds to the smaller transcript, however, the invention also relates to the polynucleotide sequence encoding a polypeptide of the 2.4 Kb transcript.

Figure 3A illustrates a hydrophobicity plot of SCM 26 and the predicted signal peptide and 7 transmembrane regions. Figure 3B shows the predicted topology of the SCM 26 protein in the membrane. Figure 3C illustrates that the SCM 26 protein is enriched in CD34<sup>+</sup> cells.

Figure 4 illustrates the nucleotide sequence of SCM 3 and corresponds to SEQ ID NO. 3. The SCM 3 cDNA contains 2990 nucleotides and ends in a poly A tail. The predicted open reading frame begins at nucleotide 82 and ends at nucleotide 1710 and encodes a protein of 543 amino acids.

Figure 5 illustrates specific features of the SCM 3 protein. The protein contains a region predicted to bind the myb factor at amino acid 72 and 9 predicted zinc-finger regions of the C2-H2 family.

Figure 6 illustrates the nucleotide and amino acid sequence of SCM 113 having 2027 nucleotides with an open reading frame from nucleotide 72 to 1889 and encoding a predicted protein of 607 amino acids (SEQ ID NO. 5).

Figure 7 illustrates sustained proliferation of genetically modified cells grown in liquid culture and incorporating a polynucleotide sequence encoding SCM 3.

#### Detailed description of the invention

Three novel cDNAs are found to be enriched in hematopoietic stem cells (HSCs). These novel genes are disclosed as SCM 26, SCM 3 and SCM 116 and are illustrated in Figures 2, 4 and 6, respectively. The term "gene" as used herein means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

The invention includes an isolated polynucleotide encoding a polypeptide having the amino acid sequence as depicted in SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, the amino acid



sequence of residues 1 – 239 of SEQ ID NO. 4, the amino acid sequence of residues 240 – 543 of SEQ ID NO. 4, and amino acid sequences functionally equivalent thereto.

The term “isolated” refers to molecules, either nucleic acids or amino acid sequences, that are removed from or separated from their original environment and are at least 60% free, preferably 75% free, more preferably 90% free and most preferably 95% free from other components with which they are naturally associated. Preferably the polypeptides and polynucleotides of the invention are purified to homogeneity.

“Nucleic acid sequence” refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin and include sense or antisense strands. A DNA “coding sequence of” or a “nucleotide sequence encoding” a particular protein, is a DNA sequence which is transcribed and translated into a protein when placed under the control of appropriate regulatory sequences. The term “polypeptide” is used interchangeably herein with the term protein.

The term “functional equivalent” is used in connection with a protein, the sequence of which is dictated by at least a part of the DNA sequences depicted in Figures 2, 4 or 6. The term means a protein having a like biological function and like or improved specific activity, and a similar amino acid sequence. “Similarity” or “identity” between two polypeptides or polynucleotides is determined by comparing the amino acid sequence and conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Similarity may be determined by procedures well known in the art, for example a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information). The present invention includes polypeptides having an amino acid sequence which is at least 75% identical to the polypeptides of SEQ ID NOs. 2, 4, 6 or fragments thereof. It is preferred that the degree of identity is at least 85%, even more preferably at least 90%, most preferably is at least 95%, still more preferably at least 97%, and most preferably at least 99% identical to a protein depicted in SEQ ID NOs. 2, 4, 6 or fragments thereof.

"Identity" as the term is used herein, refers to a polynucleotide or polypeptide sequence comprising a percentage of the same bases as a reference polynucleotide or polypeptide. For example, a polynucleotide or polypeptide which is at least 90% identical to a reference polynucleotide or polypeptide, has polynucleotide bases or amino acid residues which are identical in 90% of the bases or residues which make up the reference polynucleotide or polypeptide and may have different bases or residues in 10% of the bases or residues which comprise that polynucleotide or polypeptide sequence. One way of calculating the percentage of sequence identity is by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The term "fragment" when used in connection with an amino acid sequence means a part of the sequence depicted in Figures 2, 5 or 6 and having at least 10 amino acid residues, preferably 50 amino acids residues, even more preferably 100 amino acid residues and most preferably 200 amino acid residues which are similar to the amino acid sequences of Figures 2, 5 or 6.

A variant, i.e. a fragment polypeptide and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characters. The following non-limiting list of amino acids are considered conservative replacements (similar): a) alanine, serine, and threonine; b) glutamic acid and aspartic acid; c) asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalanine, tyrosine and tryptophan. Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

"Biological function" within the meaning of this application is to be understood in a broad sense. It includes, but is not limited to, the particular functions disclosed in this application. Further, biological functions are not only those, which a polypeptide displays in its

physiological context, i.e. as part of a living organism, but includes functions which it may perform in a non-physiological setting, e.g. in an in vitro test system. For example, a biological function of the SCM 3 gene product within the meaning of this application is the capability to improve the expansion of cells as measured in liquid culture assays as essentially described in example 5. Another example of a biological function of SCM 3 gene product within the meaning of this application is the capability to inhibit myeloid differentiation of hematopoietic progenitor cells as essentially described in example 8.

The invention includes degenerate polynucleotides, DNA sequences which encode the polypeptides of the invention and particularly the amino acid sequence of SEQ ID NOs. 2, 4, 6 and fragments thereof, but having variations in the nucleotide coding sequences. As well known in the art, the degeneracy of the genetic code allows for various nucleic acid sequences, DNA's and RNA's, to encode the same protein. In most cases an amino acid is encoded by two or more synonymous codons, for example the amino acid alanine is encoded by GCU, GCC and GCA. The invention includes polynucleotides encoding a variant of the polypeptide as shown in Figure 2, Figure 5 or Figure 6. Such nucleotide variants are alternate forms of the polynucleotide sequence which may have a deletion, substitution, or addition of one or more nucleotides and which are functionally equivalent to the encoded protein. In this context "encoding" refers to the inherent property of specific sequences of nucleotides in a nucleic acid, such as e.g. a gene in a chromosome or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having a defined sequence of nucleotides (e.g., rRNA, tRNA, other RNA molecules) or amino acids and the biological properties resulting therefrom. Thus a gene encodes a protein, if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. A nucleic acid that encodes a protein includes any nucleic acids that have different nucleotide sequences but encode the same amino acid sequence of the protein due to the degeneracy of the genetic code. Nucleic acids and nucleotide sequences that encode proteins may include introns.

An SCM gene as broadly used herein refers to the amino acid sequence of substantially purified SCM peptides obtained from any species, particularly preferred are mammalian,

including human, mouse, and chicken, and most particularly human, and from any source whether natural, synthetic or recombinant. The term SCM expression is broadly used in this disclosure to mean the expression of a polynucleotide sequence of the invention. The polypeptides so expressed are referred to as SCM proteins.

The invention further includes an isolated DNA sequence including the polynucleotide sequence of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, a fragment thereof and a sequence having at least 85% identity thereto. Isolated nucleic acid sequences are substantially similar if they are capable of hybridizing under stringent conditions to the sequence of Figures 2, 4 or 6. Isolated nucleic acid sequences are also considered substantially similar if they are polynucleotides which are at least 85%, preferably at least 90%, more preferably at least 95%, even more preferably 97%, and most preferably 99% identical to the sequences of SEQ ID NOs. 1, 3, 5 or a fragment thereof. In a preferred embodiment the fragment includes the polynucleotide encoding the amino acid sequence of residue 1 – 239 of SEQ ID NO. 4, variants and complementary sequences thereto. In another preferred embodiment the fragment includes the polynucleotide encoding the amino acid sequence of residue 240 – 543 of SEQ ID NO. 4., variants and complementary sequences thereto.

Where the term “fragment” is used with a nucleotide sequence, the term means a nucleotide sequence including part of the sequence depicted in Figures 2, 4 or 6 and comprising as few as at least 30, 50, 75, 80, 100 or more nucleotides, preferably at least 200, 300, 400, 500, 600, or more nucleotides, even more preferably at least 800, 1000, 1500, 2000 or more nucleotides. Specifically with reference to a fragment of the nucleotide sequence of Figure 2 (SCM 26), the fragment will have at least 100 nucleotides, preferably 500 nucleotides, even more preferably 800 nucleotides and most preferably at least 1000 nucleotides. Specifically with reference to a fragment of the nucleotide sequence of Figure 4 (SCM 3), the fragment will have at least 1500 nucleotides, preferably 2000 nucleotides, and most preferably at least 2500 nucleotides. Specifically with reference to a fragment of the nucleotide sequence of Figure 6 (SCM 113), the fragment will have at least 1000 nucleotides, preferably 1500 nucleotides, and most preferably at least 2000 nucleotides.

The invention further provides an isolated polynucleotide consisting of the complement of the above disclosed polynucleotides. The term complement refers to the binding of polynucleotides under permissive conditions by base pairing, for example the sequence of "AGT" binds to the complementary sequence "TCA". Most preferably a polynucleotide sequence will hybridize with the reference sequence i.e. SEQ ID NOs. 1, 3, or 6 or a part thereof under stringent conditions. Stringent hybridization conditions are those in which hybridization is effected between 50° and 60°C in saline buffer solution. The DNA to be used for hybridization may be prepared in a conventional manner and be targeted to form an identifiable probe by procedures well known in the art.

Generally, conditions may be selected that are about 5 to 20 degrees C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50 % of a target sequence hybridizes to a complementary probe. The DNA to be used for hybridization may be prepared in a conventional manner and be targeted to form an identifiable probe by procedures well known in the art.

The term antisense means nucleotide sequences that are complementary to a specific DNA or RNA sequence (sense strand). This invention further includes complementary or antisense polynucleotides.

The invention concerns isolated polypeptides which have the deduced the amino acid sequence selected from the group of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, fragments thereof, functionally equivalent polypeptides thereto. In general, a polypeptide fragment may have a sequence which is at least 10 amino acids, preferably at least 50 amino acid, even more preferably at least 100 amino acids, and most preferably at least 200 amino acids which are identical to the polypeptide sequence of Figure 2, 5, or 6.

Specific mention is made of the following preferred non-limiting polypeptide fragments: The polypeptide including amino acid residues 1 - 239 of SEQ ID NO. 4; the polypeptide including amino acid residues 240 - 543 of SEQ ID NO. 4; the polypeptide including amino acid residues 26 - 40 of SEQ ID NO. 2; the polypeptide including amino acid residues 25 - 82 of SEQ ID NO. 2; the polypeptide including amino acid residues 147 - 157 of SEQ ID NO. 2; and the polypeptide including amino acid residues 266 - 275 of SEQ ID NO. 2.

A functionally equivalent polypeptide of Figure 2, 5 or 6 is a variant wherein one in which one or more amino acid residues are substituted with conserved or non-conserved amino acid residues, or one in which one or more amino acid residues includes a substituent group. Conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among aromatic residues Phe and Tyr.

In addition, the invention features polypeptide sequences having at least 75% identity with the polypeptide sequences illustrated in Figures 2, 5, 6, or fragments and functionally equivalent polypeptides thereof. In one embodiment, the polypeptides have at least 85% identity, more preferably at least 90% identity, even more preferably at least 95% identity, still more preferably at least 97% identity, and most preferably at least 99% identity with the amino acid sequences illustrated in Figures 2, 5, 6, or the fragments including amino acid residue 1 - 239 or 240 - 543 of Figure 5.

While the SCM encoding sequence may be introduced as a construct into a host cell, in a preferred embodiment the SCM encoding sequence will be placed into a vector. The term "vector" means an agent used to carry new genes or DNA segments into cells. Vectors include the necessary elements for the transcription and translation of the inserted coding sequence. Preferred polynucleotides included in the construct or the vector are the sequences encoding for SCM 3, SCM 26, SCM 113 and functionally equivalent sequences having at least 85% identity thereto, and preferably having at least 90% identity thereto. Methods used to construct vectors are known and described in various publications. In particular techniques for constructing suitable vectors are reviewed in considerable detail in

Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Vectors may include but are not limited to viral vectors, such as baculovirus, retroviruses, adenoviruses, adeno-associated viruses, and herpes simplex viruses; bacteriophages; cosmids; plasmid vectors; synthetic vectors; and other recombination vehicles typically used in the art. In a preferred embodiment, the vector comprises a polynucleotide operatively linked to a regulatory sequence. Regulatory sequences include promoters, enhancers, polyadenylation signals, and other expression control elements. The promoter may be either a prokaryotic or eukaryotic promoter. The vector may further comprise a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid. Vectors containing both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). Specific examples include, pSG, pSV2CAT, pXt1 from Stratagene and pMSG, pSVL, pBPV and pSVK3 from Pharmacia. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate potentially extra inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Both inducible regulatory systems and constitutive regulatory sequences are known in the art to function in various cell types.

Preferred vectors include retroviral vectors (See, Coffin et al., "Retroviruses", (1997) Chapter 9 pp; 437-473 Cold Springs Harbor Laboratory Press). Vectors useful in the invention are produced recombinantly by procedures already taught in the art. WO94/29438, WO97/21824 and WO97/21825 describe the construction of retroviral packaging plasmids and packing cell lines. Exemplary vectors include the pCMV mammalian expression vectors, such as pCMV6b and pCMV6c (Chiron Corp.), pSFFV-Neo, and pBluescript-Sk+. Non-limiting examples of useful retroviral vectors are those derived from murine, avian or primate retroviruses. Common retroviruses are those based on the Moloney murine leukemia virus (MoMLV-vector). Other MoMLV derived vectors include, Lmily, LINGFER, MINGFR and MINT (Chang et al., *Blood* 92:1 - 11 (1998)). Further vectors

include those based on Gibbon ape leukemia virus (GALV) and Moloney murine sarcoma virus (MoMSV) and spleen focus forming virus (SFFV). Vectors derived from the murine stem cell virus (MESV) include MESV-MiLy (Agarwal et al., *J. of Virology*, 72:3720–3728, (1998)). Retroviral vectors also include vectors based on lentiviruses, and non-limiting examples include vectors based on human immunodeficiency virus (HIV-1 and HIV-2). New vector systems are continually being developed to take advantage of particular properties of parent retroviruses such as host range, usage of alternative cell surface receptors and the like. The present invention is not limited to particular retroviral vectors, but may include any retroviral vector. Particularly preferred vectors include DNA from a murine virus corresponding to two long terminal repeats, and a package signal. In one embodiment the murine viral vector is derived from a MoMLV or a MSCV.

In producing retroviral vector constructs, the viral gag, pol and env sequence will generally be removed from the virus, creating room for insertion of foreign DNA sequences. Genes encoded by foreign DNA are usually expressed under the control a strong viral promoter in the long terminal repeat (LTR). Selection of appropriate control regulatory sequences is dependent on the host cell used and selection is within the skill of one in the art. Numerous promoters are known in addition to the promoter of the LTR. Non-limiting examples include the phage lambda PL promoter, the human cytomegalovirus (CMV) immediate early promoter; the U3 region promoter of the Moloney Murine Sarcoma Virus (MMSV), Rous Sarcoma Virus (RSV), or Spleen Focus Forming Virus (SFFV); Granzyme A promoter; Granzyme B promoter, CD34 promoter, and the CD8 promoter. Additionally inducible or multiple control elements may be used.

Such a construct can be packed into viral particles efficiently if the *gag*, *pol* and *env* functions are provided in trans by a packaging cell line. Therefore when the vector construct is introduced into the packaging cell, the gag-pol and env proteins produced by the cell, assemble with the vector RNA to produce infectious virions that are secreted into the culture medium. The virus thus produced can infect and integrate into the DNA of the target cell, but does not produce infectious viral particles since it is lacking essential packaging sequences. Most of the packaging cell lines currently in use have been transfected with separate plasmids, each containing one of the necessary coding sequences, so that multiple recombination events are necessary before a replication competent virus can be



produced. Alternatively the packaging cell line harbors a provirus. (The DNA form of the reverse-transcribed RNA once it integrates into the genomic DNA of the infected cell). The provirus has been crippled so that although it may produce all the proteins required to assemble infectious viruses, its own RNA can not be packaged into virus. RNA produced from the recombinant virus is packaged instead. Therefore, the virus stock released from the packaging cells contains only recombinant virus. Non-limiting examples of retroviral packaging lines include PA12, PA317, PE501, PG13, ΨCRIP, RD114, GP7C-tTA-G10, ProPak-A (PPA-6), and PT67. Reference is made to Miller et al., *Mol. Cell Biol.* 6:2895 (1986); Miller et al., *Biotechniques* 7:980 (1989); Danos et al., *Proc. Natl. Acad. Sci. USA* 85:6460 (1988); Pear et al., *Proc. Natl. Acad. Sci. USA* 90:8392 –8396 (1993); Rigg et al., *Virology* 218 (1996); and Finer et al., *Blood* 83:43 – 50 (1994).

Additionally preferred vectors include adenoviral vectors (See, Frey, B.M. et al., *Blood*, 91:2781, (1998); and WO95/27071) and adeno-associated viral vectors (See, Chatterjee et al., *Current Topics in Microbiol. And Immunol.*, 218:61 – 73, 1996). Also reference is made to Shenk, Chapter 6, 161 – 178, Breakefield et al., Chapter 8 201-235; Kroner-Lux et al., Chapter 9 235 – 256 in *Stem cell Biology and Gene Therapy*, eds. Quesenberry et al., John Wiley & Sons, 1998 and U.S. Pat Nos. 5,693,531 and 5,691,176. The use of adenovirus-derived vectors may be advantageous under certain situation because they are not capable of infecting non-dividing cells, and unlike retroviral DNA, the adenoviral DNA is not integrated into the genome of the target cell. Further, the capacity to carry foreign DNA is much larger in adenoviral vectors than retroviral vectors. The adeno-associated viral vectors are another useful delivery system. The DNA of this virus may be integrated into non-dividing cells, and a number of polynucleotides have been successfully introduced into different cell types using adeno-associated viral vectors.

In one embodiment, the construct or vector will include two or more heterologous nucleic acid sequences; a) the nucleic acid sequence encoding a polypeptide of the invention and b) one or more additional nucleic acid sequence. Preferably the additional nucleic acid sequence is a polynucleotide which encodes a selective marker, a structural gene, a therapeutic gene, a ribozyme, or an antisense sequence.

A selective marker may be included in the construct or vector for the purposes of monitoring successful genetic modification and for selection of cells into which DNA has been integrated. Non-limiting examples include drug resistance markers, such as G148 or hygromycin. Additionally negative selection may be used, for example wherein the marker is the HSV-tk gene. This gene will make the cells sensitive to agents such as acyclovir and gancyclovir. Selection may also be made by using a cell surface marker, for example, to select overexpression of SCM by fluorescence activated cell sorting (FACS). The NeoR (neomycin/G148 resistance) gene is commonly used but any convenient marker gene may be used whose gene sequences are not already present in the target cell can be used. Further non-limiting examples include low-affinity Nerve Growth Factor (NGFR), enhanced fluorescent green protein (EFGP), dihydrofolate reductase gene (DHFR) the bacterial hisD gene, murine CD24 (HSA), murine CD8a(lyt), bacterial genes which confer resistance to puromycin or phleomycin, and  $\beta$ -galactosidase.

In gene therapy cells are used which express heterologous genetic material in vivo. In the case of an in born genetic disease, the genetic material is suitably a gene for the normal protein. Additionally the gene may be for a protective protein or the gene may encode a protective RNA such as a ribozyme or antisense sequence. Gene Therapy may be in vivo, administering the vector to the subject so that host target cells are transformed in situ or ex vivo wherein the target cells are transformed in vitro and then introduced into the subject.

The structural gene may be the entire gene or only the functionally active fragment of the gene. The structural gene may include for example a gene that regulates cell differentiation or a therapeutic gene capable of compensating for a deficiency in a patient that arises from a defective endogenous gene. A therapeutic gene may be one that antagonizes production or function of an infectious agent, antagonizes pathological processes, improves a host's genetic makeup, or facilitates engraftment. Specific examples of a therapeutic gene or gene sequences are ones effective in the treatment of adenosine deaminase deficiency (ADA); sickle cell anemia; recombinase deficiency; recombinase regulatory gene deficiency; HIV such as an antisense or trans-dominant REV gene or a gene carrying a herpes simplex virus thymidine kinase (HSV-tk)).

For human patients the therapeutic gene will generally be of human origin although genes of closely related species that exhibit high homology and biologically identical or equivalent function in humans may be used if the gene does not produce an adverse immune reaction in the recipient. The second polynucleotide sequence may encode new antigens or drug resistant genes or may encode a toxin or an apoptosis inducer effective to specifically kill cancerous cells, or a specific suicide gene to cancerous hematopoietic cells may be included.

A therapeutic active amount of a nucleic acid sequence or a therapeutic gene is an amount effective at dosages and for a period of time necessary to achieve the desired result. This amount may vary according to various factors including but not limited to sex, age, weight of a subject, and the like.

The additional polynucleotide sequence(s) may be introduced into the host cell on the same vector as the polynucleotide sequence encoding the polypeptides of the invention or the additional polynucleotide sequence may be introduced into the host cells on a second vector. In a preferred embodiment a selective marker will be included on the same vector as the SCM encoding nucleic acid sequence. In another embodiment, the vector will include at least three polynucleotide encoding sequences comprising the SCM encoding polynucleotide, a selectable marker and a therapeutic gene.

The host target cells of the present invention are mammalian cells and these include but are not limited to humans, mice, monkeys, farm animals, sport animals, pets, and other laboratory rodents and animals. Particularly preferred mammals are human, mice and rabbit. Preferred cells include stem cells of various cell types, such as, hematopoietic, muscle, epithelial, neural, liver, embryo and bone stem cells, particularly HSCs. Stem cells are capable of self-renewal divisions and give rise to differentiated progeny. They or their progeny contain the engrafting potential for in vivo therapeutic application. Hematopoietic stem cells are pluripotent and may also be defined in vitro by the presence of CAFC activity. General reference is made to Potten C.S. ed., *Stem Cells*, Academic Press, 1997; *Stem Cell Biology and Gene Therapy*, eds. Quesenberry et al., John Wiley & Sons Inc., 1998; and Gage et al., *Ann. Rev. Neurosci.* 18:159 – 192, 1995.

Particularly preferred host cells include hematopoietic cells. These cells encompass hematopoietic stem cells, erythrocytes, neutrophils, monocytes, platelets, mast cells, eosinophils and basophils, B and T lymphocytes and NK cells as well as the respective lineage progenitor cells. T-cells are defined as a type of lymphocyte and are thought to develop from hematopoietic stem cells. There are many types of T-cells including thymocytes, helper T-cells, inducer T-cells, suppressor T cells, or any other subset of T-cells. As used herein the term progenitor or progenitor cell indicates a cell population which no longer is a stem cell but also which has not yet become a terminally differentiated cell. The term lymphoid, myeloid, or erythroid in conjunction with the term progenitor indicates the potential cell population into which the progenitor is capable of maturing. Human hematopoietic stem cells, T-cells and lymphoid, myeloid, or erythroid progenitor cells are especially preferred host cells.

Methods of obtaining hematopoietic cells and stem cells are well known in the art and not repeated herein in any detail. In general, methods of isolating stem cells and progenitor cells include isolation from other cells in hematopoietic tissue of the body and particularly bone marrow. Stem cells and progenitor cells from bone marrow constitute only a small percentage of the total number of hematopoietic cells. Stem cells appear to be in the range of about 0.01 to about 0.1% of the bone marrow cells. Bone marrow cells may be obtained from ilium, sternum, tibiae, femora spine and other bone cavities. Other non-limiting sources of hematopoietic stem cells include embryonic yolk sac, fetal liver fetal and adult spleen, blood including adult peripheral blood and umbilical cord blood (To et al., *Blood* 89:2233 – 2258 (1997)).

For the isolation of bone marrow an appropriate solution may be used to flush the bone, including but not limited to salt solution, supplemented with fetal calf serum or other naturally occurring factors in conjunction with an acceptable buffer at low concentration, generally about 5 to 25 mM. Buffers include but are not limited to HEPES, phosphate and lactate buffers. Bone marrow can also be aspirated from the bone in accordance with conventional techniques.

The manner in which hematopoietic cells may be separated from other cells is not critical to this invention. Various procedures may be employed and include physical separation,

magnetic separation using antibody-coated magnetic beads, affinity chromatography, and cytotoxic agents joined to a monoclonal antibody or used in conjunction with a monoclonal antibody. Also included is the use of fluorescence activated cell sorters (FACS) wherein the cells can be separated on the basis of the level of staining of the particular antigens. These techniques are well known to those of ordinary skill in the art and are described in various references including U.S. Patent Nos. 5,061,620; 5,409,8213; 5,677,136; and 5,750,397; and Yau et al., *Exp. Hematol.* 18:219-222 (1990).

The order of cell separation or selection is not critical to the invention, and specific cell types may be separated either prior to genetic modification or after genetic modification.

Preferably cells are initially separated by a coarse separation followed by using positive and/or negative selection. In humans, the surface antigen expression profile of an enriched hematopoietic stem cell population may be identified by CD34<sup>+</sup>Thy-1<sup>+</sup>Lin<sup>-</sup>. Other non-limiting enriched phenotypes may include: CD2<sup>-</sup>, CD3<sup>-</sup>, CD4<sup>-</sup>, CD8<sup>-</sup>, CD10<sup>-</sup>, CD14<sup>-</sup>, CD15<sup>-</sup>, CD19<sup>-</sup>, CD20<sup>-</sup>, CD33<sup>-</sup>, CD34<sup>-</sup>, CD38<sup>low</sup>, CD45RA<sup>+</sup>, CD59<sup>++</sup>, CD71<sup>-</sup>, CDW109<sup>+</sup>, glycophorin<sup>-</sup>, AC133<sup>+</sup>, HLA-DR<sup>++</sup>, c-kit<sup>+</sup> and EM<sup>+</sup>. Lin<sup>-</sup> refers to a cell population selected on the basis of lack of expression of at least one lineage specific marker, for example CD2, CD3, CD14, and CD56. The combination of expression markers used to isolate and define an enriched HSC population may vary depending on various factors and may vary as other expression markers become available.

Murine HSCs with similar properties to the human CD34<sup>+</sup>Thy-1<sup>+</sup>Lin<sup>-</sup> may be identified by kit<sup>+</sup>Thy-1.1<sup>low</sup>Lin<sup>-lo</sup>Sca-1<sup>+</sup> (KTLS). Other phenotypes are well known. When CD34 expression is combined with selection for Thy-1, a composition comprising approximately fewer than 5% lineage committed cells can be isolated (U.S. Patent No. 5,061,620).

It has been shown CD34 is expressed on most immature T-cells also called thymocytes, and that these cells lack cell surface expression of CD1, CD2, CD3, CD4, and CD8 antigens. Also CD45RA is a useful T-cell marker. The most well-known T-cell marker is the T-cell antigen receptor (TCR). There are presently two defined types of TCRs, TCR-2 (consisting of  $\alpha$  and  $\beta$  polypeptides) and TCR-1 (consisting of  $\delta$  and  $\gamma$  polypeptides). B cells may be selected, for example, by expression of CD19 and CD20. Myeloid cells may be selected, for example, by expression of CD14, CD15, and CD16. NK cells may be selected

based on expression of CD56 and CD16. Erythrocytes may be identified by expression of glycophorin A. Compositions enriched for progenitor cells capable of differentiation into myeloid cells, dendritic cells, or lymphoid cells also include the phenotypes CD45RA<sup>+</sup>CD34<sup>+</sup>Thy-1<sup>+</sup> and CD45RA<sup>+</sup>CD10<sup>+</sup>Lin<sup>-</sup>CD34<sup>+</sup>. One skilled in the art is aware of other useful markers for various cell types.

Once the host cells are harvested and optionally separated, the cells are cultured in a suitable medium comprising a combination of growth factors that are sufficient to maintain growth. The term culturing refers to the propagation of cells on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (either morphologically, genetically or phenotypically) to the parent cell. Methods for culturing stem cells and hematopoietic cells are well known to those skilled in the art, and some of these methods are briefly mentioned herein. Any suitable culture container may be used, and these are readily available from commercial vendors. The seeding level is not critical, and it will depend on the type of cells used. In general, the seeding level will be at least 10 cells per ml, more usually at least about 100 cells per ml and generally not more than 10<sup>6</sup> cells per ml.

Various culture media can be used and non-limiting examples include Iscove's modified Dulbecco's medium (IMDM), X-vivo 15 and RPMI-1640. These are commercially available from various vendors. The formulations may be supplemented with a variety of different nutrients, growth factors, such as cytokines and the like. In general, the term cytokine refers to any one of the numerous factors that exert a variety of effects on cells, such as inducing growth and proliferation. The cytokines may be human in origin or may be derived from other species when active on the cells of interest. Included within the scope of the definition are molecules having similar biological activity to wild type or purified cytokines, for example produced by recombinant means, and molecules which bind to a cytokine factor receptor and which elicit a similar cellular response as the native cytokine factor.

The medium can be serum free or supplemented with suitable amounts of serum such as fetal calf serum, autologous serum or plasma. If cells or cellular products are to be used in humans, the medium will preferably be serum free or supplemented with autologous serum or plasma. (Lansdorp et al., *J. Exp. Med.* 175:1501 (1992) and Petzer et al., *PNAS* 93:1470 (1996)).

Non-limiting examples of compounds which may be used to supplement the culture medium are thrombopoietin (TPO), Flt3 ligand (FL), c-kit ligand (KL, also known as stem cell factor (SCF) or Stl), Interleukin (IL) such as, IL-1, IL-2, IL-3, IL-6, (soluble IL-6 receptor), IL-11, and IL-12, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage -colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), MIP-1 $\alpha$ , and erythropoietin (EPO). These compounds may be used alone or in any combination, and preferred concentration ranges may be readily determined from the published art. When murine stem cells are cultured, a preferred non-limiting medium includes mIL-3, mIL-6 and mSCF.

One skilled in the art is aware of the concentration range of these compounds in cultures. While not meant to limit the invention a general preferred range of TPO is from about 0.1ng/mL to about 500 $\mu$ g/mL, more preferred is from about 1.0ng/mL to about 1000ng/mL even more preferred is from about 5.0ng/mL to about 300ng/mL. A preferred concentration range for each of FL and KL is from about 0.1ng/mL to about 1000ng/mL, more preferred is from about 1.0ng/mL to about 500ng/mL. IL-6 is a preferred factor to be included in the culture, and a preferred concentration range is from about 0.1ng/mL to about 500ng/mL and more preferred in from about 1.0ng/mL to about 100ng/mL. Hyper IL-6, a covalent complex of IL-6 and IL-6 receptor may also be used in the culture.

Other molecules can be added to the culture media, for instance, adhesion molecules, such as fibronectin or RetroNectin™ (commercially produced by Takara Shuzo Co., Otsu Shigi, Japan). The term fibronectin refers to a glycoprotein that is found throughout the body, and its concentration is particularly high in connective tissues where it forms a complex with collagen.

In a further aspect polypeptides of the invention may be produced by culturing the host cell comprising a polynucleotide of the invention under conditions suitable for the expression of the polypeptide and recovering said polypeptide from the host culture. Methods of obtaining polypeptides from host cells grown in culture are well known in the art.

In the present invention, the methods of genetic modification are intended to encompass any genetic modification method of exogenous or foreign gene transfer or nucleic acid transfer into mammalian cells (particularly human stem cell and hematopoietic cells). The term includes but is not limited to transduction (viral mediated transfer of host DNA from a host or donor to a recipient, either in vitro or in vivo), transfection (transformation of cells with isolated viral DNA genomes), liposome mediated transfer, electroporation, calcium phosphate transfection or coprecipitation and others. Methods of transduction include direct co-culture of cells with producer cells (Bregni et al., *Blood* 80:1418 – 1422 (1992)) or culturing with viral supernatant alone with or without appropriate growth factors and polycations (Xu et al., *Exp. Hemat.* 22:223 – 230 (1994)).

In a preferred embodiment the host cells are transduced with a retroviral vector as previously described. The host cell range that may be infected is determined by the viral envelope protein. The recombinant virus can be used to infect virtually any other cell type recognized by the env protein provided by the packaging cell, resulting in the integration of the viral genome in the transduced cell and the stable incorporation of the foreign gene product. In general, murine ecotropic env of MoMLV allows infection of rodents cells, whereas amphotropic env allows infection of rodent, avian and some primate cells including human cells. Amphotropic packaging of cell lines for use with MoMLV systems are known in the art and are commercially available. These include but are not limited to, PA12, PA317,  $\psi$ CRIP, and FLYA13. (See, Miller et al., *Mol. Cell Biol.* 5:431 – 437 (1985); Mill et al., *Mol. Cell Biol.* 6:2895 – 2902 (1986); and Danos et al., *Proc.Natl. Acad. Sci. USA* 85:6460 – 6464 (1988). Recently, the G-glycoprotein from vesicular stomatitis virus (VSV-G) has been substituted for the MoMLV env protein. (See Burns et al., *Proc.Natl. Acad. Sci. USA* 90:8033-8037 (1993); and WO92/14829). Xenotropic vector systems also exist which allow infection of human cells. The genetically modified cells obtained as described above may be used immediately, expanded or frozen at for example liquid nitrogen temperatures and stored for long periods of time, being thawed and capable of being used. The cells may be stored by methods well known in the art. Once the genetically modified cells are thawed they may be further expanded. Methods of expansion of HSCs by use of growth factors and/or stromal cells associated with stem cell proliferation and differentiation are well known to those skilled in the art (US Pat. No. 5,744,361).



Methods of using the genetically modified cells include in vitro and in vivo applications. In one application, the invention further concerns, a method for increasing the effective dose of hematopoietic cells, particularly stem cells in a subject which includes obtaining a population of CD34<sup>+</sup> cells, including a subpopulation hematopoietic stem cells; introducing into the population of CD34<sup>+</sup> cells a polynucleotide sequence of the invention; obtaining a subpopulation of genetically modified stem cells which overexpress the polypeptide encoded by said polynucleotide; and administering said subpopulation of genetically modified cells to a mammalian subject wherein the effective dose of the hematopoietic stem cells is increased. Method of obtaining hematopoietic cells has previously been disclosed. The HSC may be selected using various known and previously mentioned techniques either prior to or after genetic modification. As an example the hematopoietic cells may be isolated based on phenotype expression as disclosed hereinabove. In a preferred embodiment the polynucleotide is introduced on a vector. While any method of genetic modification may be used to introduce a polynucleotide of the invention into the host cells, transduction is the preferred method of genetic modification.

An "effective amount or dose" is an amount sufficient to effect beneficial or desired results. An effective amount may be administered in one or more administrations. Determination of an effective amount is within the capability of those skilled in the art. Particularly preferred subjects of the invention in general include living mammals such as human, mice and rabbit, most preferred are humans. The administration of a genetically modified cell comprising a polynucleotide sequence of the invention may be by conventional means, for example, injection, oral administration, inhalation and others. Appropriate carries and diluents may be included in the administration of the modified cells. Samples including the modified cells and progeny thereof may be taken and tested to determine transduction efficiency. The population of CD34<sup>+</sup> cells may be cultured either prior to or after genetic modification of the host cells.

As used herein the term "overexpression" refers to expression of a polypeptide of the invention brought about by genetic modification of a host cell with a nucleic acid sequence encoding the polypeptide. Particularly preferred are polynucleotides that encode SCM 3, SCM 26, SCM 113 and functionally equivalent polypeptides having 85% identity thereto. Overexpression may take place in cells normally lacking expression of polypeptides functionally equivalent or identical to the SCM proteins claimed herein or overexpression

may take place in cells with endogenous expression of polypeptides functionally equivalent or identical to the SCM protein claimed herein. While overexpression may take place in any cell type particularly preferred host cells include hematopoietic cells, particularly HSCs and T-cells. For example, a HSC may have an endogenous level of expression of functionally equivalent or identical polypeptide to a SCM protein, but the host cell would not be genetically modified to include a nucleic acid sequence of the invention encoding a SCM polypeptide and capable of expression thereof.

As used herein a "wild type" cell is a cell type of the host cell but not genetically modified to include a polynucleotide sequence encoding a SCM polypeptide of the invention and would not result in overexpression. The overexpression of SCM proteins can be measured by various methods well known in the art. A preferred method includes the measurement of a marker gene particularly EGFP by FACS.

The method may also provide the introduction of a second polynucleotide sequence encoding a therapeutic gene, an antisense gene or a ribozyme into the population of CD34<sup>+</sup> cells as described herein above. In another application the invention concerns a method of increasing the effective dose of gene modified cells. Host cells and particularly hematopoietic stem cells overexpressing the polynucleotides of the invention are useful therapeutically. Differentiation of the cells is blocked resulting in expansion of non-differentiated stem cells. Expansion of non-differentiated stem cells gives an increase in stem cell dose either ex vivo or in vivo, thereby potentially allowing more rapid engraftment. This may result in increased representation of genetically modified cells in a subject.

The genetically modified host cells are maintained for a period of time sufficient for overexpression of SCM proteins. A suitable time period will depend *inter alia* upon cell type used and is readily determined by one skilled in the art. In general, genetically modified cells of the invention may overexpress SCM proteins for the lifetime of the host cell. Preferably, for hematopoietic cells the time period will be in the range of 1 to 45 days, more preferably in the range of 1 to 30 days, even more preferably in the range of 1 to 20 days, still more preferably in the range of 1 to 10 days, and most preferably in the range of 1 to 5 days.

A further application of the invention concerns a method of blocking the differentiation of mammalian hematopoietic stem cells including introducing a polynucleotide sequence of the invention into CD34<sup>+</sup> cells; genetically modifying a population of the CD34<sup>+</sup> cells; allowing expression of the polynucleotide sequence in the cells; and blocking differentiation of said genetically modified cells. Preferably the polynucleotide will encode SCM 3, SCM 26, SCM 113 or functionally equivalent polypeptides thereto.

As discussed above stem cells are pluripotent and capable of self-renewal. Differentiation is defined as the restriction of the potential of a cell to self renew with a change in the functional capacity of the cell. The term "blocking" differentiation is used broadly in the context of this invention and includes not only the prevention of differentiation but also means the altering of differentiation. Differentiation may be determined by methods well known in the art and these include analysis for surface markers associated with cells of a defined differentiated state. While not meant to limit the invention, generally differentiation will be slowed to about at least 10%, preferably to about 15%, more preferably to about 20%, and most preferably to about 30% or greater fewer cells expressing a specific differentiation marker. Such markers include, for example, CD4, CD8, CD13, CD14, CD19, CD36, CD40, CD41 and CD94. In a preferred embodiment, differentiation will be slowed to about at least greater than 15% and preferably greater than 20%, fewer cells expressing the marker CD14.

The method of blocking the differentiation of mammalian hematopoietic stem cells may also include isolating hematopoietic cells, particularly CD34<sup>+</sup> cells from a source of such cells and introducing a polynucleotide of the invention into the cells whereby the cells are genetically modified. Additionally it is preferred that the polynucleotide be introduced on a vector, preferably a retroviral vector wherein the host cells are genetically modified by transduced. However it is emphasized that many different vector systems as discussed above may be used in the method. Once the cells are genetically modified they are cultured in the presence of at least one cytokine in an amount sufficient to support growth of the modified cells and the modified cells are selected wherein the encoded polypeptide is overexpressed and differentiation is blocked.

The invention still further includes an antibody which binds to the polypeptides of the invention. As used herein the term SCM antibody encompasses any antibody or fragment thereof either native or recombinant, synthetic or naturally derived, which retains sufficient specificity to bind specifically to the SCM protein. The SCM antibody may be monoclonal or polyclonal that binds to SCM protein. In this regard the antibody recognizes, preferentially hematopoietic cells, particularly stem cells. For the production of antibodies, various host subjects, may be immunized by injection with SCM 3, 26 or 113 protein, or a fragment or variant thereof. General techniques for the production of antibodies are known and various protocols for measuring protein are also known including enzyme linked immunosorbant assay and fluorescent activated cell sorting. While the protein as illustrated in Figures 2, 4 or 6 may be used to raise antibodies. The protein sequence of SCM 26 is most preferred. In the present invention, a polyclonal antiserum against SCM 26 amino acid residues 26 - 40 is raised in rabbits using well known techniques. Peptide specific antibodies are purified by affinity chromatography and used for immunoblots or wildtype fibroblast or fibroblasts transfected with a retroviral vector expressing SCM 26, Figure 3. The same antibody is used to probe lysates of Liver, CD34<sup>+</sup> cells or peripheral blood leukocytes (Figure 3). Additionally extracellular regions of SCM26 may be used to generate antibodies that might be useful to detect cell surface SCM26 proteins; such fragments include amino acid sequences 25 through 82; 147 through 157 and 266 through 275 of the amino acid sequence illustrated in Figure 2.

Selecting antibodies particularly monoclonal antibodies are particularly useful for identifying markers associated with particular cell lineages and/or stages of differentiation. The antibodies may attach to a solid support to allow crude separation. The separation techniques employed should maximize the retention of viability of a fraction to be collected. Therefore, the invention encompasses not only antibodies which bind to a polypeptide of the invention but also a method of identifying stem cells, particularly hematopoietic stem cells or the progeny thereof. This includes preparing an antibody to a polypeptide of the invention, purifying the antibody, exposing a population of hematopoietic cells to the antibody allowing the exposed cells to bind with the antibody and then selecting bound cells. Techniques including antibody preparation and purification are well known in the art and these techniques are performed on a routine basis. Reference is made to *Antibodies: A Laboratory Manual*, Harlow et al., eds. (1987).

The genetically modified cells obtained by the methods herein described may be further used in an autologous or allogenic setting wherein the optionally expanded, modified cells are then used for example in bone marrow transplantation, graft facilitation, or immune reconstitution.

Furthermore various in vitro and in vivo assays are well known in the art for the measurement of the functional compositions of hematopoietic cell populations. (Reference is made to Quesenberry et al. eds., Stem Cell Biology and Gene Therapy, Wiley-Liss Inc. 1998 - Chapter 5, Hematopoietic Stem cells: Proliferation, Purification and Clinical Applications, pgs 133- 160) Non-limiting examples of these assays are briefly described herein below. The long term culture-initiating cell (LTCIC) assay involves culturing a cell population on stromal cell monolayers for approximately 5 weeks and then testing in a 2 week semisolid media culture for the frequency of clonogenic cells retained (Sutherland et al., Blood 74:1563 (1989)). The Colony-Forming Unit Culture (CFU-C) assay involves use of cell count as the number of colony-forming units per unit volume or area of a sample. The assay is used to measure clonal growth of quickly maturing progenitors in semi-solid media supplemented with serum and growth factors. Depending on the growth factors used to stimulate growth mature and/or primitive progenitors may be determined. Cobblestone area forming colony (CAFC) assays measure clonal proliferation of long-lived progenitors supported by stromal cell monolayers and growth factor/serum supplemented media. On the appropriate stromal monolayers, cells pluripotent for myeloid and lymphoid lineages may be determined. (Young et al., Blood 88:1619, (1996)). SCID-hu bone assays measure the proliferation and multilineage differentiation of cells with bone marrow repopulating activity. These cells are likely to contribute to durable engraftment in clinical transplantation. SCID-hu thymus assays measure the proliferation and differentiation in thymocytes. Both bone marrow repopulating and more mature T-lineage progenitors may be measured.

The practice of the present invention will employ, unless otherwise indicated conventional techniques of cell biology, molecular biology, cell culture, immunology and the like which are in the skill of one in the art. These techniques are fully disclosed in the current literature and reference is made specifically to Sambrook, Fritsch and Maniatis eds., "Molecular Cloning A Laboratory Manual, 2<sup>nd</sup> Ed., Cold Springs Harbor Laboratory Press, 1989); the series Methods of Enzymology (Academic Press, Inc.); and Antibodies: A Laboratory Manual, Harlow et al., eds., (1987).

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications cited herein are hereby incorporated by reference in their entirety in order to more fully describe the state of the art to which this invention pertains.

The invention generally described above will be more readily understood by reference to the following examples, which are hereby included merely for the purpose of illustration of certain embodiments of the present invention and are not intended to limit the invention in any way

## **Examples**

### **Example 1: cDNA Library Construction**

Following informed consent, human donors are treated with cyclophosphamide plus granulocyte-macrophage colony stimulating factor (GM-CSF) to mobilize CD34<sup>+</sup> Thy-1<sup>+</sup> hematopoietic stem cells (HSCs) to the peripheral blood. HSCs from multiple donors are combined. After apheresis, CD34<sup>+</sup>Thy-1<sup>+</sup>HSC stem cells are purified by flow sorting as described by Gazitt et al., *Blood*, 86:381-389 (1995). Total RNA is purified from >10<sup>7</sup> HSC using RNA-Stat (Tel-Test B inc, Friendswood, Texas). PolyA<sup>+</sup> RNA is purified from total RNA on oligo dT (Pharmacia Biotech) and used to synthesize cDNA (Stratagene unidirectional cDNA synthesis kit). Each cDNA molecule generated using this kit has an EcoR1 sticky end at the 5' end and a XhoI sticky end at the 3' end. The cDNA is directionally cloned into lambda ZAP express that had been digested with EcoR1 and XhoI restriction enzymes (Stratagene). The ligated cDNA/lambda ZAP is packaged using Gigapack III gold (Stratagene) and transfected into XL1-Blue MRF's cells (Stratagene). A total of 0.5 x 10<sup>6</sup> independent clones are produced. The lambda phage are harvested and *in vivo* excised to pBlueScript (pBS) using ExAssist helper phage and SOLR strain E. coli according to recommended Stratagene protocol.

Random clones are mini prepped by Qiagen 96 well system, restriction digestion with EcoR1 plus XhoI and electrophoresed to show inserts in the size range 0.5-5.0 Kb with an average size of 2.3 Kb. 10,000 mini prep clones are sequenced using T3 primed (i.e. 5' end) dye terminator sequencing reactions and processed on an ABI377 automated sequencer (PE Applied Biosystems). Sequence data is analyzed by BLASTX and BLASTN (Basic Local Alignment Search Tool) searches against GenBank. A number of clones are identified as being either completely novel or having homology only with ESTs.

Expression profiling is used to identify cDNA sequences which are preferentially expressed in HSCs. The cDNA inserts of clones identified as being either completely novel or having homology only with expressed sequence tags (ESTs) are amplified by PCR using T3 and T7 primers and then sent to Synteni where the microdot arrays are generated. Microdot array probes are synthesized from RNA purified from mobilized peripheral blood CD34<sup>+</sup> cells and labeled with Cy3 and from either peripheral blood cells (PBL) RNA or CD11b RNA or CD4 RNA or CD19 RNA and labeled with Cy5 using standard protocols as recommended by

Synteni. The CD34 probe and the PBL probe are mixed and allowed to hybridize to a microdot array. After hybridization and washing, the microarray is scanned to determine the intensity of probe binding to each cDNA. Hybridizations, washing and scanning are performed by Synteni. Probe binding is proportional to gene expression level. The raw binding data is balanced by monitoring probe binding to Synteni control elements on the microarray; this accounts for differences in the fluorescent labeling of the two probes. The ratio of the two binding intensities, the balanced differential expression (BDE) gives a quantitative measurement of relative gene expression level. Table 1 shows the differential expression of SCM3 in 3 independent experiments. Similar results are observed for SCM26 and SCM113 (data not shown).

**Table 1**  
**Expression profiling of SCM3 in three independent experiments.**

Experiment	CD34 Probe Value	PBL probe value (Balanced value)	Balanced differential expression (BDE)
1	10669	1944	5.5
2	21687	9271	2.3
3	10849	4681	2.3

Analysis allows the identification of 101 new cDNAs that are expressed more in stem cells than in peripheral blood cells (PBL), these cDNAs are designated selected cDNAs. The selected cDNAs are defined as being expressed at least two fold higher in stem cells (BDE>2.0) and have a low expression in PBL cells. The control cDNAs, CD34, flk2 (fetal liver kinase) and KIT (stem cell factor or alternatively steel factor, or c-Kit ligand) are known to be preferentially expressed in HSC and this is confirmed using transcript imaging.

Two approaches are taken to prioritize the 101 selected cDNAs: sequence analysis is used to confirm their new classification and further transcript imaging experiments are performed to investigate levels of expression in subsets of peripheral blood cells. Microdot arrays are analyzed with probes specific for CD34+ cells compared with either T cells (CD3<sup>+</sup>), B cells (CD19<sup>+</sup>) or myeloid cells (CD11b<sup>+</sup>). High priority cDNAs are confirmed to be novel and had



HSC-restricted expression (i.e. relatively low expression in PBL, B, T and myeloid cells). Three clones are herein identified as SCM 26, SCM 3, and SCM 113. The cDNA insert in each clone is illustrated in Figures 2, 4, and 6 and correspond to SEQ ID Nos. 1, 3 and 5.

#### Example 2: Vector Construction

cDNA inserts are subcloned from pBS and into an MSCV based retroviral vector (Hawley et al., *Gene Therapy*, 1:136 – 138 (1994)). The cDNA inserts are subcloned into vector MIE. (See Figure 1). MIE is constructed from MINGFR (Cheng et al., *Blood* 92:83-92 (1998)) by removing the nerve growth factor receptor (NGFR) gene and replacing it with enhanced green fluorescent protein (EGFP) gene on a 707bp Nco1 – blunted Bsp1 fragment. The NGFR gene is replaced by restriction digestion with ClaI, filling in the sticky end and then digestion with Nco1. The EGFP is isolated from pEGFP-1 (Clontech) and has GenBank Accession No. U55761. MIE vector has the essential components LTR-IRES-EGFP. The cDNA inserts are cloned into MIE at the EcoR1 site by PCR of the coding region of either SCM3, SCM26 and SCM113 and cloning to PCR2, removal from PCR2 by EcoR1 digestion and ligation into MIE. This gives gene expression mediated by the LTR and the ribosome entry site (IRES) allows for simultaneous translation of both the gene of interest and EGFP proteins from one primary transcript. Expression of EGFP allows selection of transduced cells by FACS.

The SCM3 cDNA fragment containing the entire coding region of SEQ ID NO. 3 is amplified by PCR and the 3' primer included an in frame hemagglutinating (HA) tag (5' TAC CCC TAC GAC GTG CCC GAC TAC GCC - SEQ ID NO. 7) followed by a stop codon, is subcloned into the MIE vector at the EcoR1 site. Additionally 3' and 5' truncations of the SCM 3 gene are made. The 5' fragment lacks the DNA binding region. The fragment is illustrated in Figure 2 from nucleotide residue 81 to nucleotide residue 783. The 3' fragment is the zinc finger domain from nucleotide residue 784 to nucleotide residue 1710. Vectors including the 5' and 3' fragments are constructed as described above for the full length SCM 3 except that for the 3' fragment the 5' PCR primer included an in frame ATG start codon. The HA tag and anti-HA antibodies are used to follow protein expression by western blots. The SCM 26 cDNA fragment containing the entire coding region of SEQ ID NO.1 is cloned out of pBS by digestion with SmaI and XhoI. MIE is digested with EcoR1, the sticky ends filled in, and then digested with XhoI. The SCM26 fragment is ligated into the blunt/XhoI cut MIE. The

SCM 113 cDNA fragment containing the entire coding region of SEQ ID NO. 5 is cloned into MIE as described above for SCM3.

### Example 3: Retroviral Infection

The retrovirus is produced by transfecting retroviral vector into the RV packaging cell line phoenix (Kinsella et al., *Human Gene Therapy*, 7(12):1405-1413, 1996) obtained from Nolan Laboratories using standard transfection protocols (Promega). Viral supernatant is collected after 48 hours.

Following informed consent, leukapheresis samples are obtained from normal adult donors mobilized with 7.5 or 10.0 µg/kg/day of granulocyte-colony stimulating factor (G-CSF) for 5 – 6 days. CD34<sup>+</sup> cells are enriched from leukapheresis samples at SyStemix (Palo Alto, CA) using Isolex 300SA or 300I (Baxter Healthcare Corp., Deerfield Ill.) as described in Young et al., *Blood*, 88:1619 – 1631, (1996), and by methods well-known in the art.

The CD34<sup>+</sup> cells are cultured at  $2 \times 10^6$  cells per ml in 10 mL cultures in serum free ex-vivo 15 medium (BioWhittaker, Walkerville, MD) for 48 hours at 37°C and 5% CO<sub>2</sub>. The cultures are supplemented with TPO, 100 ng/mL (R & D Systems, Minneapolis, MN); SCF, 100 ng/mL (SyStemix, Palo Alto CA); Flt3-L, 100 ng/mL (SyStemix, Palo Alto CA); and IL-6, 20 ng/mL as described in Luens et al., *Blood*, 91(4):1206 - 1215 (1998). After the 48 hours, the cells are centrifuged for 5 minutes at 4000 rpm at 37 °C and resuspended in the same medium described directly above. The cells are added to fibronectin fragment CH-296 (FN) (BioWhittaker, Walkerville, MD) coated plates (10 µg/mL) containing an equal volume of retroviral supernatant for 20 hour culture at 37°C in 5% CO<sub>2</sub> without polybrene or protamine sulphate. (Hananburg et al., *Human Gene Therapy*, 8:2193 – 2206, 1997). Cells are washed and incubated for an additional 72 hours with serum free ex-vivo 15 medium (BioWhittaker, Walkerville, MD) supplemented with TPO, 100 ng/mL; SCF, 100 ng/mL; Flt3-L, 100 ng/mL; and IL-6, 20 ng/mL. After incubation the CD34<sup>+</sup> cells or in some cases the Thy-1<sup>+</sup> cells expressing EGFP are purified by flow cytometry and placed into different functional assays as described below. Controls include cells transduced in parallel cultures with MIE vector containing only the EGFP.

Retroviral transduction results in constitutive stable expression of EGFP (at least for 6 weeks) with almost no decay. This is confirmed by fluorescence activated sorting (FACS) of cells following extended cultures. Expression of SCM 3 and 26 is confirmed by western blotting.

#### Example 4: Cell sorting

Cells are stained with anti-CD34-APC MoAb or isotype control.(Becton Dickinson). The staining buffer is HBSS/2% fetal calf serum (FCS) and 10 mmol/L HEPES for 20 minutes on ice together with anti-Thy-1 (GM201) PE-conjugated MoAb at 5 µg/mL. Cells are washed twice in SB and then resuspended in SB with propidium iodide (10 µg/mL. Cells are sorted on the FACSTAR Plus cell sorter (Becton Dickinson, San Jose, CA). EGFP fluorescence is detected in the FITC channel. CD34<sup>+</sup> and CD34<sup>-</sup> regions as well as Thy-1<sup>+</sup> and Thy-1<sup>-</sup> are set using the isotype controls. Cell populations from the EGFP<sup>+</sup> region are selected after removal of cells of high propidium iodide uptake and electronically gating on CD34<sup>+</sup> cells (or Thy-1<sup>+</sup> subset of CD34<sup>+</sup> cells). Reanalysis of the sorted cells indicates a purity greater than 90% for EGFP CD34<sup>+</sup>, and ranging from 60% to 95% for Thy-1<sup>+</sup> after sorting for EGFP<sup>+</sup> Thy-1<sup>+</sup> cells.

#### Example 5: Liquid Culture Assays

After sorting, cells are counted using a hemocytometer and 40,000 or 60,000 cells are incubated in ex-vivo media with TPO (100 ng/mL), SCF (100 ng/mL), FL (100 ng/mL), IL-6 (20 ng/mL) at a cell concentration of  $0.2 \times 10^6$  /mL. At days 3, 6, 10, 14, and 21 the number of alive and dead cells are counted by trypan blue exclusion. These methods are well known in the art. Cells are then plated at  $0.2 \times 10^6$  /mL.

The expression of SCM 3 has a positive effect on cell expansion after 2 weeks of culture. While overexpression of SCM 3 has little effect during the first 7 days of culture, by 14 days, cultures of SCM 3 expressing cells show enhanced viability and proliferation. This effect continues for 2 months. SCM 3 expressing cells continue to grow while control cells stopped proliferation (Figure 7). After 6 weeks of liquid culture, the number of clonogenic cells (CFU-

C) is determined (See Example 6). Cells overexpressing SCM3 are 20 fold enriched in CFU-C frequency in comparison with control cells.

#### Example 6: CFU-c Assays

To determine the effect of SCM 3, SCM 113 or SCM 26 overexpression on expansion of progenitor cells, EGFP expressing cells or control cells are sorted and placed into CFU-C assays. This assay enumerates the colonies (clonogenic cells) that grow in the presence of hematopoietic growth factors (colony stimulating factors and interleukins) from cells suspended in a semi-solid medium (methylcellulose). Enumeration of clonogenic cells (CFU-C) is a widely practiced assay for progenitor content.

CFU-C assays use MethoCult H4230 methylcellulose (Stem Cell Technologies Inc., Vancouver, Canada V5Z4J7) supplemented with IL-3 (10ng/mL); IL-6 (10ng/mL); SCF (100ng/mL); and EPO (2U/mL). Sorted cells are plated in 35mm dishes, in triplicate at 500 cells/dish. Colonies (>50 cells) are counted 14 days after plating and an average from 3 dishes are taken for each experimental condition. The colonies are classified as CFU-M (myeloid like), CFU-E (erythroid like), or CFU-Mix (mixed).

Overexpression of SCM 3 results in slightly reduced CFU-C frequency. Consistently observed in seven different experiments, is a 30% decrease in the number of CFU-M (difference significant), CFU-E (difference is not significant) and no decrease in number of more primitive CFU-MIX.

Overexpression of SCM 113 results in 40% decrease in the total CFU-C, significantly for both erythroid and myeloid lineages. Overexpression of SCM 26 results in a 25% decrease in the total number of CFU-C. Overexpression of SCM 26 also results in a decrease in number of single lineage colonies: 30% decrease in erythroid colonies, 2-fold decrease in myeloid colonies. In contrast SCM 26 overexpression gives 2-fold increase in the number of mixed type colonies in CFU-C assays (difference significant). Data not shown.

**Example 7 : Replating of CFUC to secondary colonies**

To further assess biological potential, cells from methylcellulose are harvested and plated into secondary cultures with IL-3 (10ng/mL); IL-6 (10ng/mL); SCF (100ng/mL); and EPO (2U/mL). After 14 days in culture, cells are harvested from methylcellulose by washing dishes 2x with Phosphate Buffered Saline (Dubecco), and replated at 10 000 cells /dish into methylcellulose culture, as described above. Overexpression of SCM3 increases the number of secondary colonies 4.7 fold compared to control cells. Similar results are seen with SCM 26 and SCM 113. Expression of SCM 113 increases the number of secondary colonies after replating 4.6 fold. Overexpression of SCM 26 increased replating efficiency, on average by 4.5 fold.

**Example 8: Phenotypic analysis of cells after methylcellulose or liquid culture**

After CFU-C assay and following 14 days of liquid culture, the impact of SCM 3 overexpression on myeloid differentiation is assessed using FACS analysis. Cells are harvested and stained for expression of differentiation markers (Cell surface markers, CD14, CD13 and CD33 as described in Barclay et al., *The Leucocyte Antigen Facts Book*, Academic Press, pp 132, 130,174 (1993)). Reference is also made to Becton Dickinson. Monoclonal Antibody Source Book - published by Becton Dickinson Immunocytometry Systems - San Jose, CA 95131-1807).

Expression of SCM 3 gene results in inhibition of myeloid differentiation of hematopoietic progenitor cells (Table 2). On average, overexpression of SCM 3 results in a 2-fold decrease in percentage and absolute number of cells expressing myeloid markers (CD14, Table 2 and CD13, data not shown). This is observed after 2 or 3 weeks culturing in methylcellulose in the presence of GM-CSF, IL-6, IL-3, SCF, EPO or in liquid culture in the presence of TPO, Flt3, CSF, IL-6 (difference significant). The absolute level of expression (mean of fluorescence) is also decreased (data not shown). Similar results are seen for SCM26 and SCM113.

**Table 2**  
**CD14 expression following culture.**

<b>Fold Reduction in CD14 expression after culture relative to control cells.</b>					
	<b>MIE Control</b>	<b>SCM3</b>	<b>SCM113</b>	<b>SCM26</b>	<b>SCM 3 (AA 240 –543)</b>
<b>Liquid culture</b>	<b>1</b>	<b>1.85</b>	<b>7.06</b>	<b>2.0</b>	<b>ND</b>
<b>Methyl cellulose</b>	<b>1</b>	<b>2.28</b>	<b>4.85</b>	<b>5.08</b>	<b>3.7</b>

Table 2 shows data on CD14<sup>+</sup> expression on cells with overexpression of SCM3, SCM113, SCM26 and amino acid residues 240 – 543 of SCM3. The fold reduction in CD14 expression level is shown relative to cells transduced with control MIE vector alone.

#### Example 9: SCID-bone Assays

Transduced cells as described above are injected into irradiated SCID-hu mice. The SCID bone assay is preformed as described by Murray et al., *Blood*, 85:368, 1995. C.B.-17 scid/scid mice are used as recipients of human fetal bone grafts. Limiting dilution analysis is performed to determine the dose SCM3, SCM26 or SCM113 expressing cells or control cells that will give donor reconstitution in the SCID-hu bone model. Fetal bone grafts are injected with cell doses of 5,000, 10,000, and 30,000 cells per graft into mice that receive whole body irradiation (350 rads) shortly before cell injection. Cells are not sorted for EGFP expression. At six weeks after injection the bone grafts are recovered, and the bone marrow cells are harvested and analyzed for donor cell engraftment using EGFP fluorescence and by methods well known in the art.

**Example 10: Production of SCM Antibodies.**

A polyclonal antibody to a SCM 26 fragment corresponding to amino acid residues 25 – 82 of SEQ ID No. 2. is generated and used to immunize rabbits by methods well known in the art. (Antibodies: A Laboratory Manual, Harlow et al. eds., (1987).

**Claims:**

1. An isolated polynucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of
  - a) the amino acid sequence of SEQ ID NO. 2;
  - b) the amino acid sequence of SEQ ID NO. 4;
  - c) the amino acid sequence of residues 1 - 239 of SEQ ID NO. 4;
  - d) the amino acid sequence of residues 240 - 543 of SEQ ID NO. 4;
  - e) the amino acid sequence of SEQ ID NO. 6; and
  - f) an amino acid sequence functionally equivalent to said sequence of a), b), c), d), or e).
2. The isolated polynucleotide according to claim 1 wherein the functionally equivalent amino acid sequence has at least 85% identity to said sequence of a), b), c) d) or e).
3. An isolated DNA sequence comprising a nucleotide sequence selected from the group consisting of,
  - a) the polynucleotide sequence of SEQ ID NO 1;
  - b) the polynucleotide sequence of SEQ ID NO. 3;
  - c) the polynucleotide sequence of SEQ ID NO. 5;
  - d) a fragment of the polynucleotide sequence of a), b) or c); and
  - e) a polynucleotide sequence having at least 85% identity to a polynucleotide sequence of a), b), c) or d).
4. An isolated polypeptide comprising a member selected from the group consisting of,
  - a) the amino acid sequence of SEQ ID NO. 2;
  - b) the amino acid sequence of SEQ ID NO. 4;
  - c) the amino acid sequence of residues 1 - 239 of SEQ ID NO. 4;
  - d) the amino acid sequence of residues 240 - 543 of SEQ ID NO. 4;



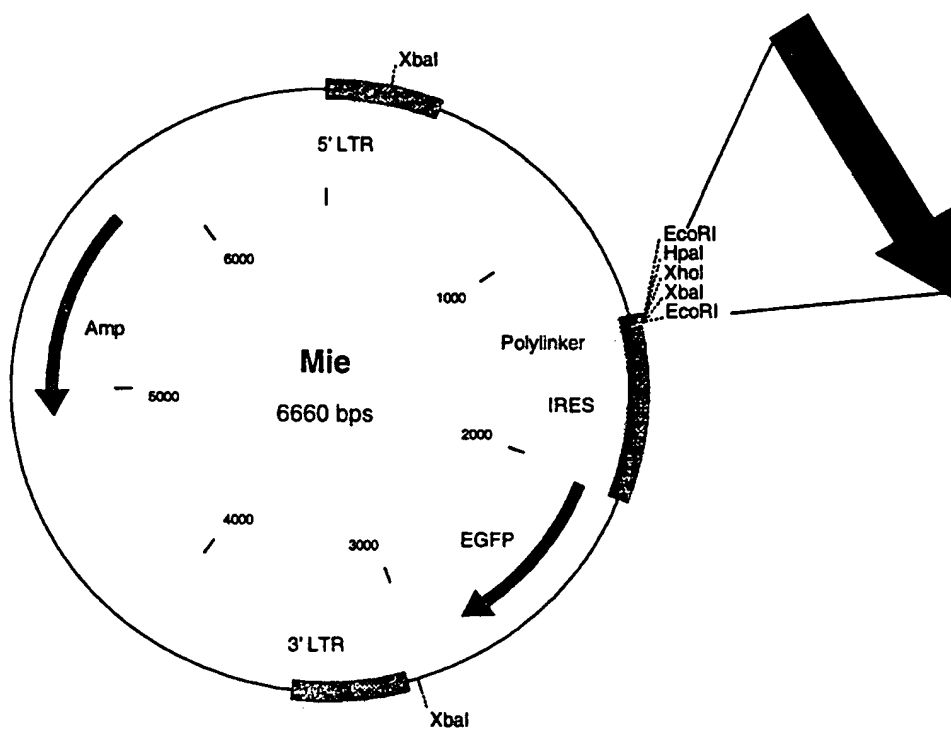
- e) the amino acid sequence of SEQ ID NO. 6; and
  - f) a polypeptide having at least 85% identity to said polypeptide of a), b), c), d) or e).
5. An isolated polypeptide comprising a member selected from the group consisting of,
- a) the amino acid residues 26 - 40 of SEQ ID NO. 2;
  - b) the amino acid residues 25 - 82 of SEQ ID NO. 2;
  - c) the amino acid residues 147 - 157 of SEQ ID NO. 2;
  - d) the amino acid residues 266 - 275 of SEQ ID NO. 2; and
  - e) a polypeptide having at least 85% identity to a), b), c) or d).
6. A vector comprising the polynucleotide sequence of any of claims 1 to 3.
7. A host cell comprising the vector of claim 6.
8. A method of increasing the effective dose of hematopoietic stem cells in a mammalian subject, comprising the steps of:
- a) obtaining a population of CD34<sup>+</sup> cells which includes a subpopulation hematopoietic stem cells;
  - b) introducing into the population of CD34<sup>+</sup> cells a polynucleotide sequence encoding a polypeptide according to claim 1 or 2;
  - c) obtaining a subpopulation of genetically modified stem cells which overexpress said polypeptide; and
  - d) administering said subpopulation of genetically modified cells to a mammalian subject wherein the effective dose of the hematopoietic stem cells is increased.
9. A method of increasing the effective dose of gene modified cells comprising the steps of;

- a) obtaining a population of hematopoietic CD34<sup>+</sup> cells which includes a subpopulation hematopoietic stem cells;
  - b) introducing into the population of CD34<sup>+</sup> cells a polynucleotide sequence encoding a polypeptide according to claim 1 or 2;
  - c) introducing into the population of CD34<sup>+</sup> cells a second polynucleotide sequence wherein said second polynucleotide sequence encodes a therapeutic gene;
  - d) obtaining genetically modified cells wherein said cells are capable of expressing the polypeptide sequence according to claim 1 and the therapeutic gene wherein the effective dose of the cells capable of expressing the therapeutic gene is increased compared to wild-type cells; and
  - e) administering the genetically modified cells to a mammalian subject.
10. A method of blocking the differentiation of mammalian hematopoietic stem cells in vitro comprising the steps of,
- a) isolating CD34<sup>+</sup> cells from a source of hematopoietic cells;
  - b) introducing a vector comprising the polynucleotide sequence of any of claims 1 or 2 into the CD34<sup>+</sup> cells whereby a population of said cells are genetically modified by said vector;
  - c) culturing the modified CD34<sup>+</sup> cells in the presence of at least one cytokine in an amount sufficient to support growth of the modified cells; and
  - d) selecting cells in which the encoded polypeptide is overexpressed whereby differentiation is blocked.
11. A method of blocking the differentiation of mammalian hematopoietic stem cells comprising the steps of,
- a) introducing a vector comprising the polynucleotide sequence of any of claims 1 or 2 into CD34<sup>+</sup> cells;
  - b) genetically modifying a population of said cells with said polynucleotide sequence;
  - c) allowing expression of said polynucleotide sequence in said cells; and

d) blocking differentiation of said genetically modified cells.

12. A method of identifying hematopoietic stem cells or progeny thereof comprising,

- a) preparing an antibody to a polypeptide of any of claims 4 or 5;
- b) purifying the antibody;
- c) exposing a population of the hematopoietic cells to the antibody;
- d) allowing said cells to bind with said antibody; and
- e) selecting said bound cells.

**Figure 1**

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Figure 2. Nucleotide sequence of SCM26

```

      10      20      30      40      50      60
CGGGGACCGA GCATTTTCAGA TCTGCTCGGT AGACCTGGTG CACCACCACC ATGTTGGCTG
                                     M L A>

      70      80      90      100     110     120
CAAGGCTGGT GTGTCTCCGG ACACTACCTT CTAGGGTTTT CCACCCAGCT TTCACCAAGG
A R L V   C L R   T L P   S R V F   H P A   F T K>

     130     140     150     160     170     180
CCTCCCCTGT TGTGAAGAAT TCCATCACGA AGAATCAATG GCTGTTAACA CCTAGCAGGG
A S P V   V K N   S I T   K N Q W   L L T   P S R>

     190     200     210     220     230     240
AATATGCCAC CAAAACAAGA ATTGGGATCC GCGTGGGAG AACTGGCCAA GAACTCAAAG
E Y A T   K T R   I G I   R R G R   T G Q   E L K>

     250     260     270     280     290     300
AGGCAGCATT GGAACCATCG ATGGAAAAAA TATTTAAAAT TGATCAGATG GGAAGATGGT
E A A L   E P S   M E K   I F K I   D Q M   G R W>

     310     320     330     340     350     360
TTGTTGCTGG AGGGGCTGCT GTTGGTCTTG GAGCATTGTG CTACTATGGC TTGGGACTGT
F V A G   G A A   V G L   G A L C   Y Y G   L G L>

     370     380     390     400     410     420
CTAATGAGAT TGGAGCTATT GAAAAGGCTG TAATTTGGCC TCAGTATGTC AAGGATAGAA
S N E I   G A I   E K A   V I W P   Q Y V   K D R>

     430     440     450     460     470     480
TTCATTCCAC CTATATGTAC TTAGCAGGGA GTATTGGTTT AACAGCTTTG TCTGCCATAG
I H S T   Y M Y   L A G   S I G L   T A L   S A I>

     490     500     510     520     530     540
CAATCAGCAG AACGCCTGTT CTCATGAACT TCATGATGAG AGGCTCTTGG GTGACAATTG
A I S R   T P V   L M N   F M M R   G S W   V T I>

     550     560     570     580     590     600
GTGTGACCTT TGCAGCCATG GTTGGAGCTG GAATGCTGGT ACGATCAATA CCATATGACC
G V T F   A A M   V G A   G M L V   R S I   P Y D>

     610     620     630     640     650     660
AGAGCCCAGG CCCAAAGCAT CTTGCTTGGT TGCTACATTC TGGTGTGATG GGTGCAGTGG
Q S P G   P K H   L A W   L L H S   G V M   G A V>

```

## FIGURE 2

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        670          680          690          700          710          720
TGGCTCCTCT GACAATATTA GGGGGTCCTC TTTCATCAG AGCTGCATGG TACACAGCTG
V A P L   T I L   G G P   L L I R   A A W   Y T A>

        730          740          750          760          770          780
GCATTGTGGG AGGCCTCTCC ACTGTGGCCA TGTGTGCGCC CAGTGAAAAG TTTCTGAACA
G I V G   G L S   T V A   M C A P   S E K   F L N>

        790          800          810          820          830          840
TGGGTGCACC CCTGGGAGTG GGCCTGGGTC TCGTCTTTGT GTCCTCAT TG GATCTATGT
M G A P   L G V   G L G   L V F V   S S L   G S M>

        850          860          870          880          890          900
TTCTTCACC TACCACCGTG GCTGGTGCCA CTCTTTACTC AGTGGCAATG TACGGTGGAT
F L P P   T T V   A G A   T L Y S   V A M   Y G G>

        910          920          930          940          950          960
TAGTTCTTTT CAGCATGTTC CTTCTGTATG ATACCCAGAA AGTAATCAAG CGTGCAGAAG
L V L F   S M F   L L Y   D T Q K   V I K   R A E>

        970          980          990          1000         1010         1020
TATCACCAAT GTATGGAGTT CAAAAATATG ATCCCATTA CTGATGCTG AGTATCTACA
V S P M   Y G V   Q K Y   D P I N   S M L   S I Y>

        1030         1040         1050         1060         1070         1080
TGGATACATT AAATATATTT ATGCGAGTTG CAACTATGCT GGCAACTGGA GGCAACAGAA
M D T L   N I F   M R V   A T M L   A T G   G N R>

        1090         1100         1110         1120         1130         1140
AGAAATGAAG TGA CT CAGCT TCTGGCTTCT CTGCTACATC AAATATCTTG TTTAATGGGG
K K>

        1150         1160         1170         1180         1190         1200
CAGATATGCA TTAAATAGTT TGTACAAGCA GCTTTCGTTG AAGTTTAGAA GATAAGAAAC

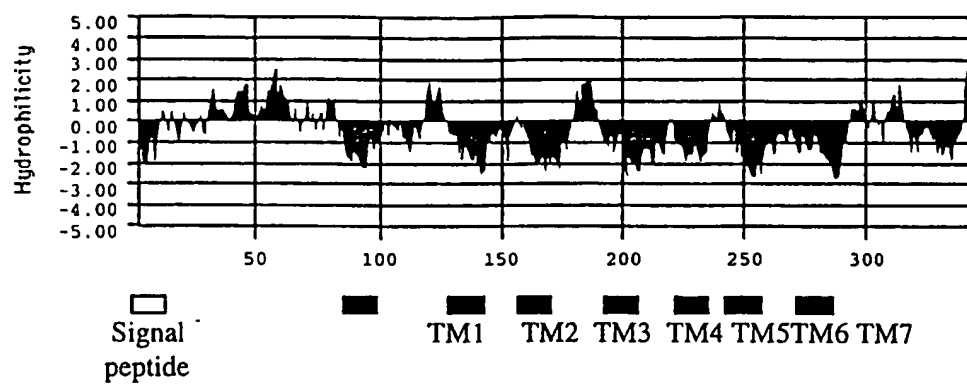
        1210         1220         1230         1240         1250         1260
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        1270         1280         1290         1300         1310
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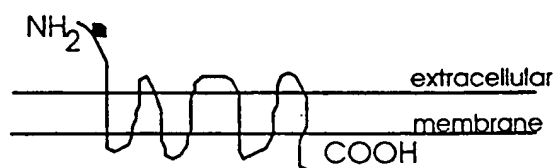
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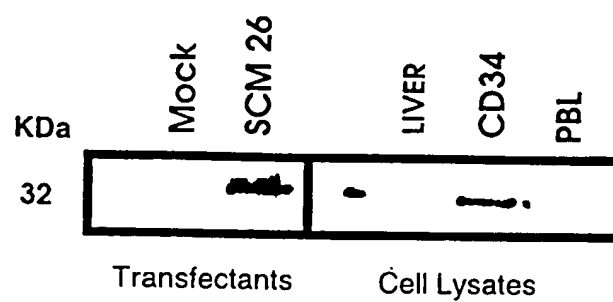
A.



B.



C.



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Figure 4. Sequence of SCM3

10	20	30	40	50	60
GTGGAGATGT	ATGCAGCATA	CAGCAGCCGC	TAGTTTTTCCT	CAGCTTCACA	TCCTGGGTGT
70	80	90	100	110	120
CGGGGGGGCTG	CCACCTTGAT	CATGGGAGTG	CCCAGTGTAG	TCAGTGCCAT	ACCTATCAGG
		M G V	P S V V	S A I	P I R>
130	140	150	160	170	180
GCAGATTGTT	CCTCCAAACC	CCAGCCCCTC	CTGCAGGGCC	AGCCTCACCT	CTACTTTTCC
A D C S	S K P	Q P L	L Q G Q	P H L	Y F S>
190	200	210	220	230	240
CCTAAGCTTT	TGTGCCAGCT	CCGGGGTTCC	TTCTTGCCCTG	TCCACTCAGC	CTGCCCTGGT
P K L L	C Q L	R G S	F L P V	H S A	C P G>
250	260	270	280	290	300
CCTCTCCTAA	CCAGGATGCC	CCAGGCAACC	ACTGTTTCTC	TGCCTTTAGG	TTCCTGGAGT
P L L T	R M P	Q A T	T V S L	P L G	S W S>
310	320	330	340	350	360
TTGACAGAGG	ATAGAGATGT	TTCTGGAGAA	TGGCCACGAG	CTTTCCCAGA	TACCCACCT
L T E D	R D V	S G E	W P R A	F P D	T P P>
370	380	390	400	410	420
GGGATGACTA	CTAGCGTCTT	CCCTGTTGCC	GGTGCCTGCC	ACAGTGTAAG	AAGCCTGCAG
G M T T	S V F	P V A	G A C H	S V K	S L Q>
430	440	450	460	470	480
AGACAACGGG	GTGCCTCCCC	ATCTCGGGAG	AGAAAACCCA	CGGGGGTGTC	GGTGATCTAC
R Q R G	A S P	S R E	R K P T	G V S	V I Y>
490	500	510	520	530	540
TGGGAGAGGC	TCCTGCTAGG	CTCAGGCAGT	GGGCAAGCCA	GCGTCAGCCT	GCGACTGACC
W E R L	L L G	S G S	G Q A S	V S L	R L T>
550	560	570	580	590	600
TCCCCGCTTA	GGCCTCCCAG	GGGCGTCCGG	CTTAGGGAAA	AGACACTCAC	AGAGCATGCG
S P L R	P P E	G V R	L R E K	T L T	E H A>
610	620	630	640	650	660
TTGCTGGGGA	GGCAGCCCAG	GACGCCTGAG	CGGCAGAAAC	CATGTGCACA	GGAGGTCCCT
L L G R	Q P R	T P E	R Q K P	C A Q	E V P>



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670 680 690 700 710 720  
 GGGAGAACCT TTGGGAGCGC CCAGGACCTG GAGGCTGCCG GCGGTCGGGG ACATCACC GA  
 G R T F G S A Q D L E A A G G R G H H R>

730 740 750 760 770 780  
 ATGGGTGCAG TTTGGCAGGA GCCTCATAGA CTCCTCGGTG GCCAGGAGCC CTCGACCTGG  
 M G A V W Q E P H R L L G G Q E P S T W>

790 800 810 820 830 840  
 GACGAGCTGG GCGAGGCTCT TCACGCTGGG GAGAAGTCCT TCGAATGCAG GGCCTGCAGC  
 D E L G E A L H A G E K S F E C R A C S>

850 860 870 880 890 900  
 AAAGTGTTTCG TGAAGAGCTC CGACCTCCTC AAGCACCTAC GCACCCACAC CGGGGAGCGG  
 K V F V K S S D L L K H L R T H T G E R>

910 920 930 940 950 960  
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 P Y E C A Q C G K A F S Q T S H L T Q H>

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 Q R I H S G E T P Y A C P V C G K A F R>

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 CATAGCTCCT CGCTGGTGCG GCACCAGCGC ATCCACACGG CCGAGAAGTC CTTCCGCTGC  
 H S S S L V R H Q R I H T A E K S F R C>

1090 1100 1110 1120 1130 1140  
 TCCGAGTGCG GCAAGGCCTT CAGCCACGGC TCCAACCTCA GCCAGCACCG CAAGATCCAC  
 S E C G K A F S H G S N L S Q H R K I H>

1150 1160 1170 1180 1190 1200  
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 A G G R P Y A C A Q C G R R F C R N S H>

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Q L L H	T G E	R P F	R C V D	C G K	A F A>
1450	1460	1470	1480	1490	1500
AAGGGCGCCG	TGCTGCTCAG	CCACCGGCGC	ATTCACACGG	GCGAGAAGCC	CTTCGTGTGT
K G A V	L L S	H R R	I H T G	E K P	F V C>
1510	1520	1530	1540	1550	1560
ACGCAGTGTG	GCCGCGCCTT	CCGTGAGCGC	CCGGCCCTCT	TCCACCACCA	GAGGATCCAT
T Q C G	R A F	R E R	P A L F	H H Q	R I H>
1570	1580	1590	1600	1610	1620
ACCGGCGAGA	AGACCGTCCG	GCGATCCAGG	GCCAGCCTGC	ACCCCCAGGC	CAGGTCTGTT
T G E K	T V R	R S R	A S L H	P Q A	R S V>
1630	1640	1650	1660	1670	1680
GCCGGGGCAT	CATCAGAAGG	TGCGCCAGCG	AAGGAAACCG	AGCCCACTCC	CGCCTCGGGC
A G A S	S E G	A P A	K E T E	P T P	A S G>
1690	1700	1710	1720	1730	1740
CCAGCCGCCG	TCTCGCAGCC	AGCGGAGGTC	TGAGGTCACA	GGTTGCAGCC	CTGGCCTTCT
P A A V	S Q P	A E V>			
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GTGAATCCCT	TCCACAGCTA	AAGGGCATAT	GTCCTCTGCA	GATCCCACAG	CAAGAAAAAG
1810	1820	1830	1840	1850	1860
TCCCGTGCTT	GCTAGTCAGG	GACAAGGAGG	CCCTTTGGCT	GTGATTTCAT	TTGCACGTGG
1870	1880	1890	1900	1910	1920
GACAGGATTT	GCCAGTTCAC	CCACAGATCA	CACCTCCATC	CCCAAAGAGG	TAGCACTGCA
1930	1940	1950	1960	1970	1980
GCAACATCAG	GGGGAGGACG	TGGTGGCTGA	ACTCTAGTGG	GGCCGAGACT	ATTCAGAGCC
1990	2000	2010	2020	2030	2040
AGTAGGAGGC	CGACAGTCAC	AGCACTGCAC	TGTGGTGCGG	CTTCATGTGA	TATGACAGTG
2050	2060	2070	2080	2090	2100
GATGCTAAGG	TGAGAGGGAT	GCAGGCATGG	GTTGGGGGTG	GCCCAGAGAA	ACTTATGACA
2110	2120	2130	2140	2150	2160
GCTGTACACA	AACTGGCCGC	TGGAGAGATG	CCCGCTGAGG	GTATTCTCCC	CTCAACCCAC

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2170	2180	2190	2200	2210	2220
TGCCTCTGTT	CATCCAAGAC	TTCCTAGGGG	CCAGCCTAGC	AGACAAGAGA	CCACAAGGGA
2230	2240	2250	2260	2270	2280
CTGGGGATCA	GGGTCCTGGG	TCTGTCAGCC	GCCACCTCTG	GGAAAGAGAA	AAGGTTTGGG
2290	2300	2310	2320	2330	2340
TCCACTGAAC	ATCATGTTTG	TAGACGCTGA	CAGGTGGGGT	CCTAATGAGA	GCCAACACAT
2350	2360	2370	2380	2390	2400
GCTCACTGCC	AGCTCCTGTC	CTGAGTACTG	GGAAGTTTCT	CCTGAAGCCC	TGTGAGATGG
2410	2420	2430	2440	2450	2460
CTCTGTGGCT	GGTATCCCGA	CTTGGAAGAT	GAGGAACTG	AGGCACACGG	CCTGGCCTGG
2470	2480	2490	2500	2510	2520
CTTCACACAC	ATAGCCGACT	CAGGAGAGGG	ATGCCCATGG	GGGAACATGT	GACTCTCAGC
2530	2540	2550	2560	2570	2580
ATTGGAAGGA	CAGAGCTAGG	ATGATGGCTT	TCCGGTGGCA	CTCGTTCAGG	TTTTTGCCCA
2590	2600	2610	2620	2630	2640
AGTCTCAGCT	TGGCCAAGGC	CTGTCACTGA	CTGGTTTACC	AAAGTCGATG	TGAGGAGGAG
2650	2660	2670	2680	2690	2700
GCTTTATACC	TGAGGGGATG	ATGTTAACTT	CAGACAAGAT	GGAGCTGCTC	ACTTTTGCCG
2710	2720	2730	2740	2750	2760
GGTTTGGTGG	CCACTTCACC	CCCAACCCTG	TCTCACCCCC	ATTATCCCTC	CTCAATTGGA
2770	2780	2790	2800	2810	2820
GGCTGGACAG	AGCTGAATAG	GAAAGACTTG	CTATTGCCTA	AGGCTATGTG	TGACACCCTC
2830	2840	2850	2860	2870	2880
CTGAGGACCT	CCCCACCCCA	GTGTAATGGC	CCTTCATGGC	AGGGACAGAA	AGGTGGACTG
2890	2900	2910	2920	2930	2940
GGGGCCATTT	GCTTCCTGTG	GCCTTCAGCA	GACCAGGCC	TGTCCCTACC	TGGAGCCTCA
2950	2960	2970	2980	2990	
CCTCCAAGGA	AATTCATGTT	CTCCTTAATG	GAAAAAAAAA	AAAAAAAAAA	AA

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Figure 5. Features of SCM3 protein:

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      10      20      30      40      50      60
MGVPSVVSAL PIRADCSSKP QPLLQGQPHL YFSPKLLCQL RGSFLPVHSA CPGPLLTRMP

      >MybDNA_bind
      70      80      90      100      110      120
QATTVSLPLG SWSLTEDRDV SGEWPRAFPD TPPGMTTSVF PVAGACHSVK SLQRQRGASP

      130      140      150      160      170      180
SRERKPTGVS VIYWERLLLG SGSGQASVSL RLTSPLRPPE GVRLREKTLT EHALLGRQPR

      190      200      210      220      230      240
TPERQKPCAQ EVPGRTFGSA QDLEAAGGRG HHRMGAVWQE PHRLLGQEP STWDELGEAL

      >ZINC1      >ZINC1
      250      260      270      280      290      300
HAGEKSFECR ACSVFVKSS DLLKHLRTH GERPYECAQC GKAFSQTSHL TQHQRIHSGE

      >ZINC1      >ZINC1
      310      320      330      340      350      360
TPYACPVCCK AFRHSSSLVR HQRIHTAEKS FRCSECGKAF SHGSNLSQHR KIHAGGRPYA

>ZINC1      >ZINC1      >ZINC1
      370      380      390      400      410      420
CAQCGRRFCR NSHLIQHRT HTGEKPFVCA LCGAAFSQGS SLFKHQRVHT GEKPFACPQC

      >ZINC1      >ZINC1
      430      440      450      460      470      480
GRAFSHSSNL TQHQLLHTGE RPFRCVDCGK AFAKGAVLLS HRRHTGEKP FVCTQCGRF

      490      500      510      520      530      540
RERPALFHQ RIHTGEKTVR RSRASLHPQA RSVAGASSEG APAKETETPT ASGPAAVSQP

AEV

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Figure 6. Sequence of SCM113

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      10      20      30      40      50      60
CTTGGAGTGA GTGGACGCAC TCGGGAATTG TAGGAGGACG AGGCTCAGCT CTTGCCAGGC

      70      80      90     100     110     120
CAAATTGAGA CATGTCTGAC ACAAGCGAGA GTGGTGCAGG TCTAACTCGC TTCCAGGCTG
      M S D   T S E S   G A G   L T R   F Q A>

      130     140     150     160     170     180
AAGCTTCAGA AAAGGACAGT AGCTCGATGA TGCAGACTCT GTTGACAGTG ACCCAGAATG
E A S E   K D S   S S M   M Q T L   L T V   T Q N>

      190     200     210     220     230     240
TGGAGGTCCC AGAGACACCG AAGGCCTCAA AGGCACTGGA GGTCTCAGAG GATGTGAAGG
V E V P   E T P   K A S   K A L E   V S E   D V K>

      250     260     270     280     290     300
TCTCAAAGC CTCTGGGGTC TCAAAGGCCA CAGAGGTCTC AAAGACCCCA GAGGCTCGGG
V S K A   S G V   S K A   T E V S   K T P   E A R>

      310     320     330     340     350     360
AGGCACCTGC CACCCAGGCC TCGTCTACTA CTCAGCTGAC TGATACCCAG GTTCTGGCAG
E A P A   T Q A   S S T   T Q L T   D T Q   V L A>

      370     380     390     400     410     420
CTGAAAACAA GAGTCTAGCA GCTGACACCA AGAAACAGAA TGCTGACCCG CAGGCTGTGA
A E N K   S L A   A D T   K K Q N   A D P   Q A V>

      430     440     450     460     470     480
CAATGCCTGC CACTGAGACC AAAAAGGTCA GCCATGTGGC TGATACGAAG GTCAATACAA
T M P A   T E T   K K V   S H V A   D T K   V N T>

      490     500     510     520     530     540
AGGCTCAGGA GACTGAGGCT GCACCCTCTC AGGCCCCAGC AGATGAACCT GAGCCTGAGA
K A Q E   T E A   A P S   Q A P A   D E P   E P E>

      550     560     570     580     590     600
GTGCAGCTGC CCAGTCTCAG GAGAATCAGG ATACTCGGCC CAAGGTCAA GCCAAGAAAG
S A A A   Q S Q   E N Q   D T R P   K V K   A K K>

      610     620     630     640     650     660
CCCGAAAGGT GAAGCATCTG GATGGGGAAG AGGATGGCAG CAGTGATCAG AGTCAGGCTT
A R K V   K H L   D G E   E D G S   S D Q   S Q A>

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670	680	690	700	710	720
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S G T T	G G R	R V S	K A L M	A S M	A R R>
730	740	750	760	770	780
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A S R G	P I A	F W A	R R A S	R T R	V G C>
790	800	810	820	830	840
TGGGCCCCGA	GAGCCTTGCT	CTCCTGAGAT	CACCTAAAGC	CCGTAGGGGC	AAGGCTCGCC
L G P E	S L A	L L R	S P K A	R R G	K A R>
850	860	870	880	890	900
GTAAGCTGC	CAAGCTCCAG	TCATCCCAAG	AGCCTGAAGC	ACCACCACCT	CGGGATGTGG
R R A A	K L Q	S S Q	E P E A	P P P	R D V>
910	920	930	940	950	960
CCCTTTTGCA	AGGGAGGGCA	AATGATTTGG	TGAAGTACCT	TTTGGCTAAA	GACCAGACGA
A L L Q	G R A	N D L	V K Y L	L A K	D Q T>
970	980	990	1000	1010	1020
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K I P I	K R S	D M L	K D I I	K E Y	T D V>
1030	1040	1050	1060	1070	1080
ACCCCGAAAT	CATTGAACGA	GCAGGCTATT	CTTTGGAGAA	GGTATTTGGG	ATTCAATTGA
Y P E I	I E R	A G Y	S L E K	V F G	I Q L>
1090	1100	1110	1120	1130	1140
AGGAAATTGA	TAAGAATGAC	CACTTGTCAC	TTCTTCTCAG	CACCTTAGAG	CCCACTGATG
K E I D	K N D	H L Y	I L L S	T L E	P T D>
1150	1160	1170	1180	1190	1200
CAGGCATACT	GGGAACGACT	AAGGACTCAC	CCAAGCTGGG	TCTGCTCATG	GTGCTTCTTA
A G I L	G T T	K D S	P K L G	L L M	V L L>
1210	1220	1230	1240	1250	1260
GCATCATCTT	CATGAATGGA	AATCGGTCCA	GTGAGGCTGT	CATCTGGGAG	GTGCTGCGCA
S I I F	M N G	N R S	S E A V	I W E	V L R>
1270	1280	1290	1300	1310	1320
AGTTGGGGCT	GCGCCCTGGG	ATACATCATT	CACCTTTTGG	GGACGTGAAG	AAGCTCATCA
K L G L	R P G	I H H	S L F G	D V K	K L I>
1330	1340	1350	1360	1370	1380
CTGATGAGGT	TGTGAAGCAG	AAGTACCTGG	ACTATGCCAG	AGTCCCCAAT	AGCAATCCCC
T D E V	V K Q	K Y L	D Y A R	V P N	S N P>

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P E Y E F F W G L R S Y Y E T S K M K V>

1450 1460 1470 1480 1490 1500  
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L K F A C K V Q K K D P K E W A A Q Y R>

1510 1520 1530 1540 1550 1560  
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E A M E A D L K A A A E A A A E A K A R>

1570 1580 1590 1600 1610 1620  
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A E I R A R M G I G L G S E N A A G P C>

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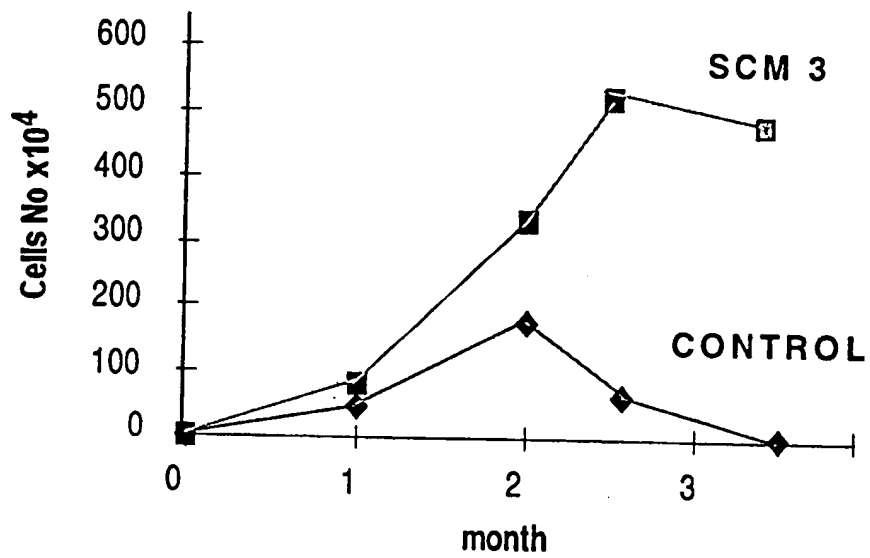
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Figure 7





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Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala Leu

85 90 95

Cys Tyr Tyr Gly Leu Gly Leu Ser Asn Glu Ile Gly Ala Ile Glu Lys

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Ile Leu Gly Gly Pro Leu Leu Ile Arg Ala Ala Trp Tyr Thr Ala Gly  
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Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu Lys  
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Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val Phe  
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Val Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Thr Val Ala Gly  
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Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe Ser  
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Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu Val  
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Ser Pro Met Tyr Gly Val Gln Lys Tyr Asp Pro Ile Asn Ser Met Leu  
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Pro Ile Arg Ala Asp Cys Ser Ser Lys Pro Gln Pro Leu Leu Gln Gly  
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cag cct cac ctc tac ttt tcc cct aag ctt ttg tgc cag ctc cgg ggt 207  
Gln Pro His Leu Tyr Phe Ser Pro Lys Leu Leu Cys Gln Leu Arg Gly  
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Ser Phe Leu Pro Val His Ser Ala Cys Pro Gly Pro Leu Leu Thr Arg
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      45              50              55
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atg ccc cag gca acc act gtt tct ctg cct tta ggt tcc tgg agt ttg 303
Met Pro Gln Ala Thr Thr Val Ser Leu Pro Leu Gly Ser Trp Ser Leu
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      60              65              70
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aca gag gat aga gat gtt tct gga gaa tgg cca cga gct ttc cca gat 351
Thr Glu Asp Arg Asp Val Ser Gly Glu Trp Pro Arg Ala Phe Pro Asp
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acc cca cct ggg atg act act agc gtc ttc cct gtt gcc ggt gcc tgc 399
Thr Pro Pro Gly Met Thr Thr Ser Val Phe Pro Val Ala Gly Ala Cys
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cac agt gta aaa agc ctg cag aga caa cgg ggt gcc tcc cca tct cgg 447
His Ser Val Lys Ser Leu Gln Arg Gln Arg Gly Ala Ser Pro Ser Arg
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gag aga aaa ccc acg ggg gtg tcg gtg atc tac tgg gag agg ctc ctg 495
Glu Arg Lys Pro Thr Gly Val Ser Val Ile Tyr Trp Glu Arg Leu Leu
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      125              130              135
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cta ggc tca ggc agt ggg caa gcc agc gtc agc ctg cga ctg acc tcc 543
Leu Gly Ser Gly Ser Gly Gln Ala Ser Val Ser Leu Arg Leu Thr Ser
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      140              145              150
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ccg ctt agg cct ccc gag ggc gtc cgg ctt agg gaa aag aca ctc aca 591
Pro Leu Arg Pro Pro Glu Gly Val Arg Leu Arg Glu Lys Thr Leu Thr
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gag cat gcg ttg ctg ggg agg cag ccc agg acg cct gag cgg cag aaa 639
Glu His Ala Leu Leu Gly Arg Gln Pro Arg Thr Pro Glu Arg Gln Lys
175          180          185

cca tgt gca cag gag gtc cct ggg aga acc ttt ggg agc gcc cag gac 687
Pro Cys Ala Gln Glu Val Pro Gly Arg Thr Phe Gly Ser Ala Gln Asp
190          195          200

ctg gag gct gcc ggc ggt cgg gga cat cac cga atg ggt gca gtt tgg 735
Leu Glu Ala Ala Gly Gly Arg Gly His His Arg Met Gly Ala Val Trp
205          210          215

cag gag cct cat aga ctc ctc ggt ggc cag gag ccc tcg acc tgg gac 783
Gln Glu Pro His Arg Leu Leu Gly Gly Gln Glu Pro Ser Thr Trp Asp
220          225          230

gag ctg ggc gag gct ctt cac gct ggg gag aag tcc ttc gaa tgc agg 831
Glu Leu Gly Glu Ala Leu His Ala Gly Glu Lys Ser Phe Glu Cys Arg
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gcg tgc agc aaa gtg ttc gtg aag agc tcc gac ctc ctc aag cac cta 879
Ala Cys Ser Lys Val Phe Val Lys Ser Ser Asp Leu Leu Lys His Leu
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cgc acc cac acc ggg gag cgg ccc tac gag tgc gcc cag tgc ggc aag 927
Arg Thr His Thr Gly Glu Arg Pro Tyr Glu Cys Ala Gln Cys Gly Lys

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gcc ttc agc cag acg tcg cac ttg acg cag cac cag cgc atc cac agc   975
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      285              290              295
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ggc gag acg ccc tac gcg tgc ccc gtg tgc ggc aag gcc ttc cgg cat   1023
Gly Glu Thr Pro Tyr Ala Cys Pro Val Cys Gly Lys Ala Phe Arg His
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agc tcc tcg ctg gtg cgg cac cag cgc atc cac acg gcc gag aag tcc   1071
Ser Ser Ser Leu Val Arg His Gln Arg Ile His Thr Ala Glu Lys Ser
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ttc cgc tgc tcc gag tgc ggc aag gcc ttc agc cac ggc tcc aac ctc   1119
Phe Arg Cys Ser Glu Cys Gly Lys Ala Phe Ser His Gly Ser Asn Leu
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agc cag cac cgc aag atc cac gcg ggt ggg cgt cct tat gct tgc gca   1167
Ser Gln His Arg Lys Ile His Ala Gly Gly Arg Pro Tyr Ala Cys Ala
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cag tgt ggc cgc cgc ttc tgc cgc aac tcg cac ctg atc cag cac gag   1215
Gln Cys Gly Arg Arg Phe Cys Arg Asn Ser His Leu Ile Gln His Glu
      365              370              375
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cgt acg cac aca ggc gag aag ccc ttc gtg tgc gcg ctc tgc ggt gct   1263
Arg Thr His Thr Gly Glu Lys Pro Phe Val Cys Ala Leu Cys Gly Ala
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Ala Phe Ser Gln Gly Ser Ser Leu Phe Lys His Gln Arg Val His Thr
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ggc gag aag ccc ttc gcc tgc cca cag tgc ggc cgc gcc ttt agc cac 1359
Gly Glu Lys Pro Phe Ala Cys Pro Gln Cys Gly Arg Ala Phe Ser His
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agc tcc aac ctc acc cag cac cag ctc ctg cac acg ggc gag cgg ccc 1407
Ser Ser Asn Leu Thr Gln His Gln Leu Leu His Thr Gly Glu Arg Pro
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Phe Arg Cys Val Asp Cys Gly Lys Ala Phe Ala Lys Gly Ala Val Leu
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ctc agc cac cgg cgc att cac acg ggc gag aag ccc ttc gtg tgt acg 1503
Leu Ser His Arg Arg Ile His Thr Gly Glu Lys Pro Phe Val Cys Thr
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cag tgt ggc cgc gcc ttc cgt gag cgc ccg gcc ctc ttc cac cac cag 1551
Gln Cys Gly Arg Ala Phe Arg Glu Arg Pro Ala Leu Phe His His Gln
475          480          485          490
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agg atc cat acc ggc gag aag acc gtc cgg cga tcc agg gcc agc ctg 1599
Arg Ile His Thr Gly Glu Lys Thr Val Arg Arg Ser Arg Ala Ser Leu
"          495          500          505
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cac ccc cag gcc agg tct gtt gcc ggg gca tca tca gaa ggt gcg cca 1647
His Pro Gln Ala Arg Ser Val Ala Gly Ala Ser Ser Glu Gly Ala Pro
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510 515 520

gcg aag gaa acc gag ccc act ccc gcc tgc gcc cca gcc gcc gtc tgc 1695  
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525 530 535

cag cca gcg gag gtc tgaggtcaca ggttcagacc ctggccttct gtgaatccct 1750  
Gln Pro Ala Glu Val

540

tccacagcta aagggcatat gtcctctgca gatccacag caagaaaaag tcccgtgctt 1810

gctagtcagg gacaaggagg ccctttggct gtgatttcat ttgcacgtgg gacaggattt 1870

gccagttcac ccacagatca cacctccatc cccaagagg tagcactgca gcaacatcag 1930

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Ser Pro Lys Leu Leu Cys Gln Leu Arg Gly Ser Phe Leu Pro Val His
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~           35           40           45
~
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Ser Ala Cys Pro Gly Pro Leu Leu Thr Arg Met Pro Gln Ala Thr Thr
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~           50           55           60
~
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Val Ser Leu Pro Leu Gly Ser Trp Ser Leu Thr Glu Asp Arg Asp Val
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~           65           70           75           80
~
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Ser Gly Glu Trp Pro Arg Ala Phe Pro Asp Thr Pro Pro Gly Met Thr
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~           85           90           95
~
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Thr Ser Val Phe Pro Val Ala Gly Ala Cys His Ser Val Lys Ser Leu
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Gln Arg Gln Arg Gly Ala Ser Pro Ser Arg Glu Arg Lys Pro Thr Gly
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Val Ser Val Ile Tyr Trp Glu Arg Leu Leu Leu Gly Ser Gly Ser Gly
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Gln Ala Ser Val Ser Leu Arg Leu Thr Ser Pro Leu Arg Pro Pro Glu
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Gly Val Arg Leu Arg Glu Lys Thr Leu Thr Glu His Ala Leu Leu Gly
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" Arg Gly His His Arg Met Gly Ala Val Trp Gln Glu Pro His Arg Leu  
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" Leu Gly Gly Gln Glu Pro Ser Thr Trp Asp Glu Leu Gly Glu Ala Leu  
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" His Ala Gly Glu Lys Ser Phe Glu Cys Arg Ala Cys Ser Lys Val Phe  
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" Val Lys Ser Ser Asp Leu Leu Lys His Leu Arg Thr His Thr Gly Glu  
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" Arg Pro Tyr Glu Cys Ala Gln Cys Gly Lys Ala Phe Ser Gln Thr Ser  
" 275 280 285  
" His Leu Thr Gln His Gln Arg Ile His Ser Gly Glu Thr Pro Tyr Ala  
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" Cys Pro Val Cys Gly Lys Ala Phe Arg His Ser Ser Ser Leu Val Arg  
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" His Gln Arg Ile His Thr Ala Glu Lys Ser Phe Arg Cys Ser Glu Cys

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" His Ala Gly Gly Arg Pro Tyr Ala Cys Ala Gln Cys Gly Arg Arg Phe  
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" Cys Arg Asn Ser His Leu Ile Gln His Glu Arg Thr His Thr Gly Glu  
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" Lys Pro Phe Val Cys Ala Leu Cys Gly Ala Ala Phe Ser Gln Gly Ser  
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Lys Thr Val Arg Arg Ser Arg Ala Ser Leu His Pro Gln Ala Arg Ser

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Gly Val Ser Lys Ala Thr Glu Val Ser Lys Thr Pro Glu Ala Arg Glu
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"

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" 175 180 185  
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" agc agt gat cag agt cag gct tct gga acc aca ggt ggc cga agg gtc 686  
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cgc tcg gac atg ctg aag gac atc atc aaa gaa tac act gat gtg tac   1022
Arg Ser Asp Met Leu Lys Asp Ile Ile Lys Glu Tyr Thr Asp Val Tyr
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ccc gaa atc att gaa cga gca ggc tat tct ttg gag aag gta ttt ggg   1070
Pro Glu Ile Ile Glu Arg Ala Gly Tyr Ser Leu Glu Lys Val Phe Gly
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Trp Ala Lys Ala Arg Ile Gln Ala Gly Ala Glu Ala Lys Ala Lys Ala  
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caa gag agt ggc agt gcc agc act ggt gcc agt acc agt acc aat aac 1742  
Gln Glu Ser Gly Ser Ala Ser Thr Gly Ala Ser Thr Ser Thr Asn Asn  
545 550 555  
agt gcc agt gcc agt gcc agc acc agt ggt ggc ttc agt gct ggt gcc 1790  
Ser Ala Ser Ala Ser Ala Ser Thr Ser Gly Gly Phe Ser Ala Gly Ala  
560 565 570  
agc ctg acc gcc act ctc aca ttt ggg ctc ttc gct ggc ctt ggt gga 1838  
Ser Leu Thr Ala Thr Leu Thr Phe Gly Leu Phe Ala Gly Leu Gly Gly  
575 580 585  
gct ggt gcc agc acc agt ggc agc tct ggt gcc tgt ggt ttc tcc tac 1886  
Ala Gly Ala Ser Thr Ser Gly Ser Ser Gly Ala Cys Gly Phe Ser Tyr  
590 595 600 605  
aag tga gattttagat attgttaatc ctgccagtct ttctcttcaa gccaggggtgc 1942  
Lys  
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Ala Glu Asn Lys Ser Leu Ala Ala Asp Thr Lys Lys Gln Asn Ala Asp  
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Pro Gln Ala Val Thr Met Pro Ala Thr Glu Thr Lys Lys Val Ser His  
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Val Ala Asp Thr Lys Val Asn Thr Lys Ala Gln Glu Thr Glu Ala Ala  
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Pro Ser Gln Ala Pro Ala Asp Glu Pro Glu Pro Glu Ser Ala Ala Ala  
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Gln Ser Gln Glu Asn Gln Asp Thr Arg Pro Lys Val Lys Ala Lys Lys  
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Ala Arg Lys Val Lys His Leu Asp Gly Glu Glu Asp Gly Ser Ser Asp  
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Gln Ser Gln Ala Ser Gly Thr Thr Gly Gly Arg Arg Val Ser Lys Ala  
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Leu Met Ala Ser Met Ala Arg Arg Ala Ser Arg Gly Pro Ile Ala Phe  
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Trp Ala Arg Arg Ala Ser Arg Thr Arg Val Gly Cys Leu Gly Pro Glu  
" 225 230 235 240  
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Ser Leu Ala Leu Leu Arg Ser Pro Lys Ala Arg Arg Gly Lys Ala Arg  
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Arg Arg Ala Ala Lys Leu Gln Ser Ser Gln Glu Pro Glu Ala Pro Pro  
" 260 265 270  
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Pro Arg Asp Val Ala Leu Leu Gln Gly Arg Ala Asn Asp Leu Val Lys
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Glu Pro Thr Asp Ala Gly Ile Leu Gly Thr Thr Lys Asp Ser Pro Lys
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Leu Gly Leu Leu Met Val Leu Leu Ser Ile Ile Phe Met Asn Gly Asn
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Arg Pro Gly Ile His His Ser Leu Phe Gly Asp Val Lys Lys Leu Ile
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